ON THE FORMATION OF SUGARS FROM FORMALDEHYDE

A Thesis submitted to the University of Glasgow

for the Degree of Doctor of Philosophy

in the Faculty of Science

by

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A C K N O W L E D G M E N T S

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Thanks are also due to Dr P. Ingram and Miss Helen Fullerton for supplying and identifying the rocks used in Chapter 5. I gratefully acknowledge the sugar samples supplied by Dr A.G. Cairns Smith, Dr P. Ingram and Mr R.B. Walker.

The Science Research Council provided a grant for this work.

Finally, I must thank my wife for her help in drawing some of the diagrams, and not least, for her ever-lasting patience.

SUMMARY

This Thesis is concerned principally with the formose reaction, the conversion of formaldehyde to sugars: it begins with an historical account. The various catalysts, accelerators products are tabulated and the kinetic characteristics reviewed. A number of mechanisms that have been proposed at one time or another, are discussed in the context of whether they refer to the first part of the reaction (the initial formation of glycolaldehyde) or to the second (the creation of higher sugars). The aldol and benzoin condensation, and the Cannizzaro reactions, which have relevance to the formose reaction, are briefly mentioned.

An examination of the alumina-catalysed formose reaction follows this. Such a system has been used to elucidate the behaviour of some of the accelerators. In place of the earlier suggestions that "active formaldehyde" was produced by such accelerators as benzoin and vitamin C, new mechanisms have been proposed. The minimum requirement for sugar accelerators has been shown to be the possession of the hydroxyacetyl group. In addition, a new type of accelerator has been found: this has the minimum requirement of a methylene α-diketone or a benzoyl methyl group. e.g. diacetyl and kojic acid. The two classes of accelerator coincide in benzoyl carbinol.

While neutral and basic alumina catalysed the formose reaction, acidic alumina did not. As this was presumably a pH effect, investigations were undertaken with solutions of various pH to determine at what pH sugar formation became significant. For experimental reasons preliminary investigations were carried out in sodium hydroxide solution, which is homogeneous at these pHs. The change to sugar was very sudden, so sudden that a "switch on" parameter could be assigned to it. It was in solutions of the highest pH that sugars were formed: immediately below this

the final pHs after a prolonged reaction time were approximately constant, probably because of a bufferring effect caused by the Cannizzaro reaction. In solutions of the lowest initial pH no changes were observed. Highly charged ions formed sugars at lower pHs. The tetramethylammonium ion seemed not to favour the Cannizzaro reaction, instead permitting sugar formation at a much lower pH than would have been expected on comparison with other monovalent ions. This observation strongly supports the hypothesis that co-ordination to cations is not necessary for formaldehyde's conversion to sugars: the effect of cyanide ions on this system reinforces this.

The reduction of carbon dioxide to formaldehyde photochemically has also been reviewed. Much uncertainty is attached to the validity of earlier claims, although more recent work suggests that the aldehyde is actually formed.

The possibility of the formose reaction on the primitive earth was entertained. Starting with the components of the primitive atmosphere, the formation of formaldehyde was reviewed, and the local formation of organic compounds under such circumstances considered (with special reference to formaldehyde). The formose reaction has been shown to be catalysed by rocks. Other factors affecting it have also been mentioned.

The formose system comprises a complex, dynamic set of reactions. Speculations on such a system being a primitive phenotype under the influence of a clay genetic material are made.

C O N T E N T S

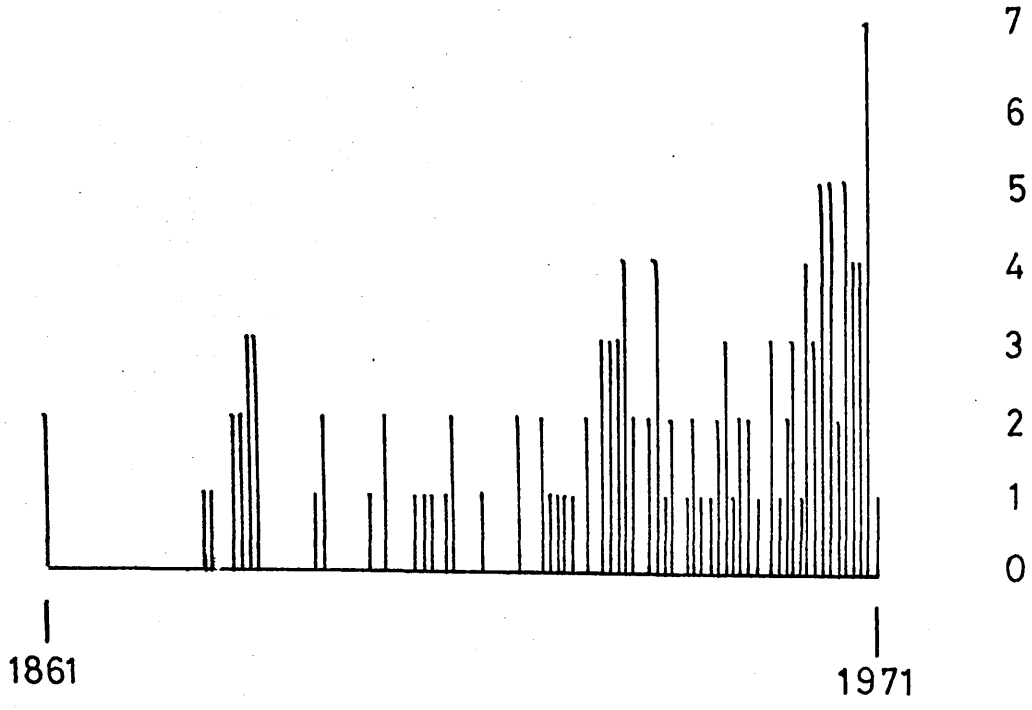
"Sugar" is a generic term which includes both mono- and polysaccharides. The "sugars" which will be encountered most often in the following pages are monosaccharides - which have been defined[1] as polyhydroxyaldehydes or ketones with at least three aliphatically bound carbon atoms. This definition excludes the first product of the formose reaction, glycolaldehyde, which has two carbon atoms.

The definition of "sugar" will be extended to include glycolaldehyde.

Fig. I

NUMBER OF PAPERS PUBLISHED ANNUALLY

ON THE FORMOSE REACTION



CHAPTER 1

THE FORMOSE REACTION

INTRODUCTION

The conversion of formaldehyde into sugars has been known for over a century[2]. First discovered accidentally, the "formose reaction" has provided sufficient variety and complexity to merit more than one hundred papers with, no doubt, many more to follow. Interest in the reaction has been fairly constant during this time (Fig.1), and has not been confined only to chemists. At one time it was thought that the reaction might have been involved in photosynthesis in plants: this has now been discounted. Re search is at present in progress to determine whether the formose reaction could be incorporated into the closed-circuit respiratory systems that will have to be used in prolonged spaceflight by Man. It may also have occurred on the primitive earth.

Because of the large volume of work this review has been split into a number of sections which describe the catalysts, accelerators, intermediates, products and the kinetics and mechanism of the reaction. Related reactions, such as the aldol and benzoin condensations, the Cannizzaro reaction and caramelisation of sugars,are also mentioned. Further sections deal with the patents that involve formose, the use of formose as a food and, in a later chapter, its participation in studies on the origin of life. A number of smaller reviews may be found elsewhere.[42,104,106,107,126]

First of all we shall summarise the relevant chemical properties of formaldehyde.

THE RELEVANT CHEMICAL PROPERTIES OF FORMALDEHYDE

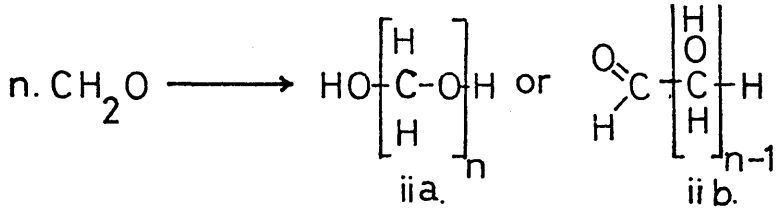
i Aqueous solutions of formaldehyde.

In concentrated aqueous solution (up to 40%) formaldehyde exists as a mixture of polyoxymethylene glycols[3]. Dilution reduces the average degree of polymerisation until the single-carbon unit, methylene glycol, predominates. The concentration of unhydrated formaldehyde in aqueous solution is very low, at best one molecule in twelve hundred[4]. Dissolution of anhydrous formaldehyde in water results in an exothermic hydration of the molecule, involving the liberation of 15 kilocalories per gram mole[5].

In alcoholic solution hemiacetal formation occurs[6].

ii Polymer formation.

Formaldehyde may polymerise in two ways - by carbon-oxygen bond formation or carbon-carbon bond formation.



ii a. ii b.

a Carbon-oxygen bond formation.

Polyoxymethylene glycols are formed by this process, representing the structure of formaldehyde in the solid e.g. paraformaldehyde, and in concentrated aqueous solution[7]. The chemical properties exhibited by these polymers are those that would be expected of formaldehyde alone. Although depolymerisation occurs easily, especially in the presence of strong acid or base[8], account must be taken of the rate of the process when kinetic studies are being undertaken.

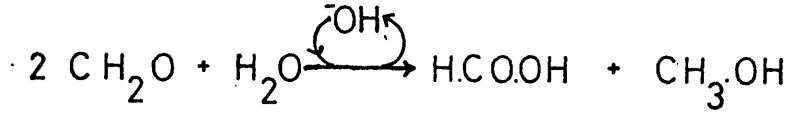
b Carbon-carbon bond formation.

This results in polyhydroxyaldehydes and ketones and is the subject of this review.

Because of these two types of "polymerisation", a distinction is often made in terminology. Formaldehyde "polymers" are polyoxymethylene glycols: polyhydroxyaldehydes and -ketones, sugars, arise from formaldehyde "condensation".

iii The Cannizzaro reaction.

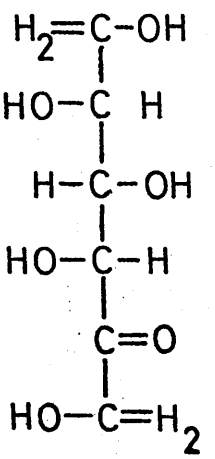
The disproportionation of formaldehyde, a Cannizzaro reaction, often accompanies sugar formation. Equimolar quantities of formic acid and methanol result. Further mention of this reaction will be found in Appendix IV.



A detailed account of the physical and chemical properties of formaldehyde will be found in J.F.Walker's monograph.*

* J.F. Walker, "Formaldehyde", 3rd. Edition. American Chemical Society Monograph No. 159: Reinhold Publishing Corporation, 1964.

LOEW'S FORMULA FOR FORMOSE



THE DISCOVERY AND EARLY DEVELOPMENT OF THE FORMOSE REACTION

The formation of sugars from formaldehyde was first reported by A. Butlerow, a Russian chemist, in 1861.[2] Two years before that he had announced his discovery of formaldehyde and was engaged in the investigations of its chemical properties.[9] His attempt to hydrolyse methylene diacetate with boiling lime-water resulted in the formation of a brown liquid with a burned odour and a bitter taste. It possessed some of the properties of sugars. When formaldehyde itself replaced the diacetate a very similar substance was obtained: Butlerow called this "methylenitan".[2]

When the reaction was carried out at a lower temperature the product was different. It was colourless, sweet-tasting and analysed exactly for sugar.[11,12] On heating, however, it was transformed into the brown substance with the burned odour. This difference was demonstrated by Loew, who subsequently claimed the distinction of being the first to isolate sugars from formaldehyde condensation, not their decomposition products.[35]

Fifteen years elapsed between Butlerow and Loew's work, but in the next fifteen there was an average of more than one paper per year. Experimental activity was directed towards increasing the number of catalysts for the reaction and towards elucidating the constitution of the product. Lead,[11] zinc,[12,27] tin,[15,18] magnesium oxide and hydroxide,[15,26,19,21] barium oxide,[10] lead hydroxide,[22,23] calcium carbonate,[25] and sodium sulphite[24] were all catalytically active, and various names were applied to the products - "formose",[11,13,14] "acrose",[17] "pseudoformose",[18] and "methose".[18,21,26] The giving of such names reflects the opinion of the early investigators that individual compounds were being formed. Indeed it is not uncommon to find melting point temperatures of osazone and other derivatives in the literature.[11,12,15,41] Loew suggested that a formula for formose might be that

on the opposite page.

The similarity of formose, acrose, methylenitan soon became apparent[16,17,20] and E. Fischer concluded that methylenitan and formose were in principle identical.[20] He suggested that the term "formose" should be used to describe the sugars obtained from formaldehyde condensation.[16] This name is still in use.

Formose is usually a mixture of sugars containing between two and six carbon atoms, but occasionally individual sugars predominate. This may explain why earlier workers were able to isolate sharply-melting crystalline derivatives. Both diacetylosazones and methyl glyoxalosazones, derivatives of the decomposition products of sugar, were characterised as well.[28]

FORMOSE REACTION CATALYSTS

The Catalysts.

The number of catalysts for the formose reaction has been greatly extended, involving both organic and inorganic compounds (Table I.).

Inorganic catalysts have been known for the longest time and are, in general, basic salts - often the oxides, hydroxides and carbonates of Group I to IV metals. The first attempts to find out exactly what was causing the reaction were made by Schmalfuss in 1927.[39] After examining a number of combinations of cations and anions, he concluded that the cation (of magnesium) was at the seat of the activity.

The cation is not the sole catalytic agent: the hydroxide ion is also important.[82] A difference can also be found between mono- and divalent cations. In solutions of potassium ions bufferred to the pH of calcium and lead hydroxides, the condensation took much longer than when the divalent ions were present.[48,51] The addition of neutral salts with the same cation as the catalyst had a rate enhancing effect, while addition of salts of those cations which were less effective catalysts had the opposite effect.[39,82]

Malinowski and co-workers tried in vain to extend the list of active oxides.[94,99] Al_2O_3, TiO_2, V_2O_5, CrO_3, MnO_2, ZnO, SrO, MoO_3, Ag_2O, CdO, SnO, SnO_2, Sb_2O_3, BaO, WO_3, HgO, Bi_2O_3 and ThO_2 were devoid of activity. Since then alumina has been used by myself and others to produce formose.[119] Malinowski has also varying claims about the feasibility of using magnesium oxide.[94,99] Part of the reason for this discrepancy may lie in the pH of the solution in which the reaction occurred. We shall come to other reasons later in this Thesis.

Table I summarises the elements that have been used as possible formose catalysts. Table II records the cations involved.

TABLE I. FORMOSE REACTION CATALYSTS

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE*	REFERENCE
1861	Butlerow	$Ca(OH)_2$	+	2
1882	Tollens	BaO	+	10
1886	Loew	Pb	+	11
1886	Loew	Zn	+	12
1888	Loew	MgO	+	15
1888	Loew	Sn	+	15,18
1889	Loew	$Mg(OH)_2$	+	19
1897	Loew	$Mg(OH)_2$	+	21
1899	de Bruyn, van Ekenstein	$Pb(OH)_2$	+	22,23
1904	Seywetz, Gibello	Na_2SO_3	+	24
1906	Euler, Euler	$CaCO_3$	+	25
1906	Loeb	MgO	+	26
1908	Loeb	Zn	+	27
1909	Loeb	KOH, Zn, $ZnCO_3$	+	28,29
		Fe	-	
1910	Loeb, Pulvermacher	$Pb(OH)_2$	+	30
1914	Franzen	$Sr(OH)_2$	+	33
1915	Franzen, Hauck	$Ba(OH)_2$	+	34
1919	Ewart	NaOH + salts of Ca, Ba, Sr	+	36

* Where formose has been identified a "+" appears, and where none has been formed "-".

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCE
1924	Schmalfuss Kalle	MgO	+	37
1924	Kuster, Schoder	$MgSO_4$, $CaCO_3$, $CaHPO_4$, $Pb(OH)_2$	+	38
1927	Schmalfuss	NaOH	+	39,43
	Congehl	$NaOOCH$, $KOOCH$	-	
		$MgCl_2$, MgO	+	
		$Mg(OOCH)_2$, $Mg(OAc)_2$	+	
		$Ca(OH)_2$, $Ca(OOCH)_2$	+	
		$Cu(OOCH)_2$	-	
		$Al(OH)_3$, Al_2O_3	-	
1928	Metsre	NaOH	+	40
1928	Vogel	MgO, PbO, $Ca(OH)_2$	+	41
1931	Karrer, Krauss	CaO	+	44
1933	Orthner, Gerisch	NaOH, $Pb(OH)_2$	+	46
1933	Gorr, Wagner	unknown	+	47
1935	Kuzin	MgO, $Ca(OH)_2$, $Pb(OH)_2$	+	48
		calcium fructosate	+	
		$KOH-K_2HPO_4$ pH 12	-	
		12	+	
		12.6	+	
		14.9	+	
1935	Kuzin	$Ca(OH)_2$	+	49
1935	Kuzin	NaOH, $Ca(OH)_2$	+	50
1936	Kuzin	NaOH, $Ca(OH)_2$	+	51
1936	West, Ney	$Ca(OH)_2$	+	52
1937	Balezin	$Ca(OH)_2$	+	54

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCE
1937	Kuzin	$Ca(OH)_2$?	+	56
1938	Kuzin	Mg(OH)(OAc) at pH 8.2	+	57
1938	Kuzin	$Ca(OH)_2$	+	58
1938	Prudhomme	alkaline earth oxides	+	60
1939	Taylor, et al.	oxides or hydroxides of Mg, Ca, Sr, Ba, Sn, Pb	+	61
1940	Handford Schreiber	compounds of Groups II, IV	+	62
1942	Langenbeck	$Ca(OH)_2$	+	63,70
1942	Handford Schreiber	Sn, Pb compounds	+	64
1942	Lorand	oxides, hydroxides, carbonates organic salts of lead, zinc, alkaline earths: uncleaned strips of lead	+	66
1942	Hunig	$NaOH-CaCl_2$	+	67
1943	Schoenemann	promoters	+	68
1944	Katzschmann	unknown	+	69
1946	Balezin	CaO	+	71
1947	Balezin	CaO	+	72
1947	Cornubert Peyrade	$Ca(OH)_2/CaCO_3$ mixtures	+	73
1948	Langenbeck	Pb, PbO	+	74
1950	Cornubert Peyrade	$Ca(OH)_2$, $CaCO_3$ calcite, aragonite	+ +	76

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCE
1950	Cornubert	BaO, $BaCO_3$(witherites)	+	77
	Peyrade	$CaCO_3$	+	
1951	Pfeil	TlOH	+	78
1951	Cornubert	$CaCO_3$	+	79
	Peyrade			
1951	Schlussel	CaO (and Tesla coil)	+	80
	Machery			
1951	Hough, Jones	$Ca(OH)_2$	+	81
1951	Pfeil, Schroth	LiOH, NaOH, TlOH	+	82
		$Ca(OH)_2$, $Ba(OH)_2$	+	
1953	Mariani,	PbO	+	83
	Torraca			
1954	Langenbeck	$Pb(OH)_2$	+	85,86
1956	MacLean, Heinz	$Mg(OH)_2$, $Ca(OH)_2$, $Ba(OH)_2$	+	72
		$Sn(OOCH)_2$	+	
		PbO, $PbNO_3$, $Pb(OAc)_2$	+	
		Pb(OH)(OAc)	+	
1956	Langenbeck,	ion-exchange resins with	+	88
	Kruger, Welker,	active groups		
	Schwarzer			
1957	Langenbeck,	ion-exchange resins	+	89
	Schwarzer			
1957	Balezin	$Ca(OH)_2$	+	90
	Surikina			
1959	Binko, Kolar	MgO	+	92

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCE
1959	Breslow	NaOH, KOH, TlOH	+	93
		$Ca(OH)_2$, $Pb(OH)_2$	+	
1960	Malinowski,	MgO, CaO	+	94
	Kehl, Tyrlik	Al_2O_3, MnO_2, ZnO, CdO, HgO	-	
		SnO, SnO_2, Sb_2O_3, Bi_2O_3	-	
		Et_3PbOH, $Et_2Pb(OH)_2$, Et_2PbO	-	
1960	Mayer, Jaschke	CaO, $CaCO_3$, PbO	+	95,96
1961	Pfeil, Ruckert	$Ca(OH)_2$	+	97
1962	Malinowski,	TiO_2, V_2O_5, CrO_3, MoO_3, Ag_2O	-	99
	Kehl, Gora	SrO, BaO, WO_3, ThO_2, MgO	-	
		Pb + 1% CaO	+	
		PbO + (MgO, BaO, CuO, ZnO, CrO_3)	-	
1962	Imyanitov	Et_3N	+	100
1963	Akerlof, Mitchell	$Me_4N.OH$	-	105
1964	Sinyak	unknown	+	108
1964	Akerlof	alkaline conditions	+	110
1964	Orestov	CaO	+	111
1965	Runge, Mayer	pyridine, picolines, collidines	+	114
1965	Ruckert, Pfeil, Scharf	$Ca(OH)_2$	+	116
1965	Ito	CaO	+	116
1966	Balezin, Surikina	$Mg(OH)_2$, $Ca(OH)_2$, $Sr(OH)_2$	+	117
		$Ba(OH)_2$, $Pb(OH)_2$	+	
1966	Orestov	$Ca(OH)_2$	+	118

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCES
1967	Gabel, Ponnamperuma	alumina, kaolinite, illite	+	119
1967	Reid, Orgel	carbonate-apatite mixture	+	120
		calcium carbonate	+	
1967	Verbrugge	alkaline solution	+	121
1967	Runge, Mayer	NaOH, TlOH, $Ca(OH)_2$, $Ba(OH)_2$	+	122
		$Pb(OH)_2$, $BaCO_3$, $CaCO_3$	+	
		pyridine, picolines, collidines	+	
		diethylaminoethanol	+	
		N-methylmorpholine/piperidine	+	
1967	Shapira	lead, copper, zinc, iron oxides	+	123
1968	Weiss, Shapira	$Ca(OH)_2$	+	124
1968	Reisz	zeolites and γ-irradiation	+	125
1969	Nakai, Tsujigado, Sato	$Ca(OH)_2$	+	128
1969	Glotova, Enikolopyan	KOH-$Ca(OH)_2$	+	131
1970	Shapira	Al_2O_3 + (CaO, PbO, Fe_2O_3)	+	130,132
1970	Shapira, Weiss, La Pierre	$Ca(OH)_2$	+	133
1970	Sinyak	rare earth hydroxide	+	134
1970	Mizuno, Mori, Shiomi, Nakatsujii	NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$	+	135
		$Sr(OH)_2$, $Ba(OH)_2$, $Pb(OH)_2$	+	
		TlOH, $Me_4N.OH$	+	
		pyridine, picolines, collidines	+	
		triethanolamine,	+	
		2-(dimethylamino)ethanol	+	
		N-methylmorpholine/piperidine	+	

TABLE I cont.

YEAR	INVESTIGATOR(S)	CATALYST(S)	FORMOSE	REFERENCE
1970	Glotova	calcium gluconate	+	136
1970	Krylov, Sinyak, Uspenskaya, Shul'gina	$Ca(OH)_2$, $Sr(OH)_2$	+	137
1970	Chermside, Grandez, Shapira, Furst	unknown	+	138
1971	Berlin, Krylov, Sinyak	hydroxides of La, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yt $Ce(OH)_4$, $Th(OH)_4$	+	148

TABLE II

CATIONS OF INORGANIC FORMOSE CATALYSTS EXCLUDING ELEMENTAL FORMS

H																	He
Li^{I}	Be											B	C	N	O	F	Ne
Na^{I}	Mg^{II}											Al^{III}	Si	P	S	Cl	Ar
K^{I}	Ca^{II}	Sc	Ti^{IV}	V^{V}	Cr^{VI}	Mn^{IV}	Fe	Co	Ni	Cu	Zn^{II}	Ga	Ge	As	Se	Br	Kr
Rb	Sr^{II}	Y	Zr	Nb	Mo^{VI}	Tc	Ru	Rh	Pd	Ag^{I}	Cd^{II}	In	Sn^{II}	Sb^{III}	Te	I	Xe
Cs^{I}	Ba^{II}	+	Hf	Ta	W^{VI}	Re	Os	Ir	Pt	Au	Hg^{II}	Tl^{I}	Pb^{II}	Bi^{III}	Po	At	Rn
Fr	Ra	++															

+	$La^{?}$	Ce^{IV}	Pr	Nd	Pm	Sm^{III}	Eu	$Gd^{?}$	$Tb^{?}$	$Dy^{?}$	Ho^{III}	$Er^{?}$	$Tm^{?}$	$Yb^{?}$	Lu
++	Ac	Th^{IV}	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md		

Me_4N^+

formose

no formose

not attempted

TABLE III

ORGANIC CATALYSTS FOR THE FORMOSE REACTION

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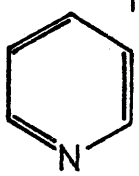

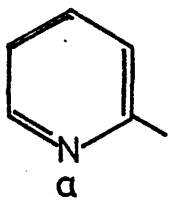

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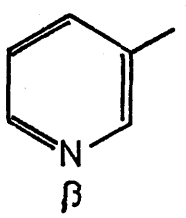

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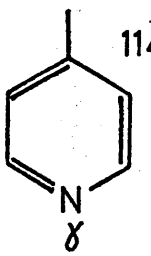

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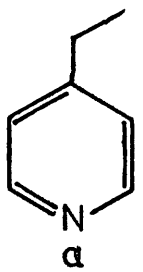

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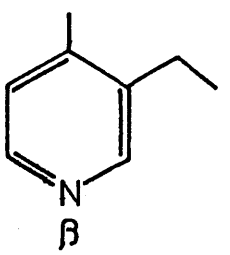

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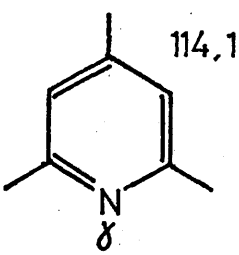

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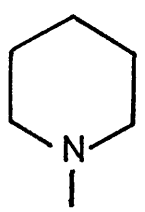
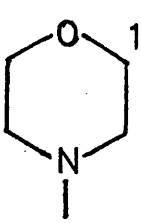




122 135

$(CH_3CH_2)_3N$[100]

$Et_2N.CH_2.CH_2OH$[122]

$(HO.CH_2CH_2)_3N$[122]

$Me_2N.CH_2CH_2OH$[122]

"Tertiary amines"[135]

The elemental forms of magnesium, zinc, tin and lead have been mentioned as formose catalysts. The participation of surface oxide would seem a more likely basis for the activity, in view of the number of catalysts of that type in Table I. "Uncleaned strips of lead" have been recommended as good catalysts in one industrial process.[66]

The formose reaction may be catalysed in homogenous or heterogenous solution.[133,119] In the latter case the total amount of catalyst may greatly exceed the amount of formaldehyde *e.g.* in the alumina-catalysed reaction 5.0g Al_2O_3 were needed for 250mg CH_2O. Ion exchange resins have been used to advantage,[88,89] as have mixed catalysts, such as CaO-PbO,[99] $CaO-Al_2O_3$, $PbO-Al_2O_3$, $Fe_2O_3-Al_2O_3$.[130,132] A few naturally occurring materials also cause formaldehyde condensation to occur - calcite,[73,76,77,79,120] baryta,[77] witherites,[77] kaolinite and illite.[119]

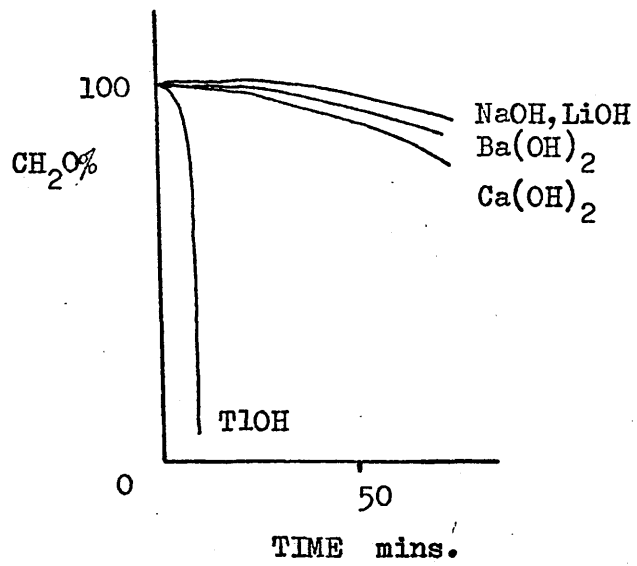
Anions have not been studied intensively, except by Schmalfuss,[39] but it would seem that hydroxide ion is the only one involved. (We shall mention this ion later.) Seywetz's finding[24] that sodium sulphite was a catalyst may be explained by the presence of a sodium hydroxide impurity.

Ammonium hydroxide and formaldehyde form hexamethylenetetramine when added together and, consequently, no mention is found of its catalytic nature. Tetramethylammonium hydroxide has been found both to catalyse and not to catalyse the condensation.[135,105] Nitrogen-containing formose catalysts are otherwise organic compounds.

The principal advantage of the use of organic bases has been in the suppression of the Cannizzaro reaction, which often accompanies sugar formation in the presence of inorganic catalysts.[114,122] Degradation of the sugars once they have been formed is also reduced.

A summary of the organic catalysts is given in Table III.

THE CONSUMPTION OF FORMALDEHYDE UNDER THE ACTION OF VARIOUS HYDROXIDES



(After Pfeil and Schroth[82])

The Choice of a Catalyst.

What is a "good" catalyst for the formose reaction? Lime is the catalyst that has been most often used, but it is not necessarily the best catalyst because of this. The answer must, of course, depend on the criteria used to decide what is "good". It may be that the largest yield of sugar is sought, or, perhaps, the fastest rate of sugar formation, or the lowest temperature at which sugars can be made, or the ability to preserve the sugars once they have been formed.

Pfeil has given a list of cations in decreasing order of the rate of sugar formation:[82]

$$Tl^{I} \rangle\ Ca^{II} \rangle\ Ba^{II} \rangle\ Na^{I},\ Li^{I}.$$

Mizuno has arranged then in order of the yield of sugar:[135]

$$Ca^{II} \rangle\ Ba^{II} \rangle\ Sr^{II} \rangle\ Mg^{II} \rangle\ Pb^{II} \rangle\ K^{I},\ Na^{I},\ Tl^{I}.$$

We can see that the order in the two lists is the same except for thallium. Thallium hydroxide therefore forms sugars very quickly, but also destroys them. The involvement of the various ions will be mentioned later.

Solvents.

Before we end the section on catalysts it is perhaps appropriate to mention the solvents for the formose reaction. The most common solvent is water, but alcohols have been used. The principal advantage of alcohols seems to be their ability to reduce the extent of the Cannizzaro reaction (Appendix V). Anhydrous conditions have been used successfully, but a protic solvent was still necessary (Appendix V).

Finally, the oxides, hydroxides and carbonates of Mg^{II}, Ca^{II}, Sr^{II}, and Ba^{II}, and the oxides and hydroxides of aluminium in the presence or absence of a tertiary amine are excellent catalysts - for the vapour phase polymerisation of formaldehyde: they are the subject of a Japanese patent.[139] May they have anhydrous conditions!

TABLE IV. FORMOSE REACTION ACCELERATORS

YEAR	INVESTIGATOR(S)	ACCELERATOR(S)	EFFECT*	REFERENCE
1910	Loeb, Pulvermacher	sugar	+	30
1927	Schmalfuss, Congehl	sugar (glucose, fructose)	+	39,43
		methanol	0	
		acids	-	
		furfural + acid	-	
1933	Gorr, Wagner	glycerol, ethylene glycol	+	47
		methanol, ethanol	+	
1935	Kuzin	calcium fructosate	+	48
		fructose + $Ca(OH)_2$	+	
		fructose + $Pb(OH)_2$	+	
		mannitol	0	
		glycerol	0	
		saccharose	0	
		glycolaldehyde	+	
		glucose	+	
		maltose	+	
		penta-O-acetylfructose	0	
1935	Kuzin	acetoneglucose	0	49
		3,5,6-trimethylglucose	+	
		3,5,6-trimethylacetoneglucose	0	
		benzoin	+	
		diphenyl or benzil (?)	0	
		acetoin	+	
		acetone	0	
		ethyl acetoacetate	0	
		ethoxyacetaldehyde	+	

* Where an acceleration has been observed a "+" appears, where no effect has been found "0", and where inhibition has been experienced "-".

TABLE IV cont.

YEAR	INVESTIGATOR(S)	ACCELERATOR(S)	EFFECT	REFERENCE
1935	Kuzin	α-hydroxymethylbenzoin	+	50
1936	Kuzin	glucose + NaOH	+	51
		glucose + $Ca(OH)_2$	+	
1936	West, Ney	ascorbic acid	+	52
1937	Balezin	glucose, fructose	+	54
1937	Kuzin	ascorbic acid	+	55
		iso-ascorbic acid	+	
1938	Kuzin	glycolaldehyde	+	57
		fructose	+	
		ascorbic acid	+	
1938	Kuzin	glucose	+	58
1940	Handford, Schreiber	enediols	+	62,64
1942	Langenbeck	glycolaldehyde	+	63,70
		dihydroxyacetone	+	
		glucose	+	
		fructose	+	
		benzoin	+	
		α-hydroxymethylbenzoin	+	
		anisoin	+	
		acetoin	+	
1943	Hunig	glycolaldehyde	+	67
		reductone	+	
		p-nitrotoluene	-	
1943	Schoenemann, Apel, Berger	formose	+	68

TABLE IV cont.

YEAR	INVESTIGATOR(S)	ACCELERATOR(S)	EFFECT	REFERENCE
1946	Balezin	glucose	+	71,72
1948	Langenbeck	benzoyl carbinol (?)	+	74
1949	Langenbeck,	benzoyl carbinol	+	75
	Sander, Hunig,	naphthoyl carbinol	+	
	Katzschmann	acenaphthoyl carbinol	+	
		monohydroxyacetone	+	
		dihydroxyacetone	+	
		glycolaldehyde	+	
		glucose	+	
		fructose	+	
1951	Cornubert,	dihydroxyacetone	+	79
	Peyrade			
1952	Pfeil, Schroth	glucose (small amount)	+	82
		glucose (large amount)	-	
		isopropanol	+	
		methanol	+	
		glycerol	-	
		ethylene glycol (small amt.)	+	
		ethylene glycol (large amt.)	-	
		dioxane	+	
		tetrahydrofuran	+	
1954	Langenbeck	benzoyl carbinol + $Ca(OH)_2$	+	86
		benzoyl carbinol + $Pb(OH)_2$	+	
		benzoyl carbinol + cyanide	+	
1956	MacLean, Heinz	glucose	+	87
		formose	+	

TABLE IV cont.

YEAR	INVESTIGATOR(S)	ACCELERATOR(S)	EFFECT	REFERENCE
1956	Langenbeck, Kruger, Schwarzer, Welker	derivatives on ion-exchange resins:- p-phenetidine, aniline morpholine acetamide benzamide (poly-aminostyrene resin)	+	88
1957	Langenbeck, Schwarzer	ion-exchange resins with $-CO.CH_2OH$ and $-CO.CH_2.NR_1R_2$ groups, where R_i is alkyl, aryl or H.	+	89
1959	Breslow	m-, p-cyanobenzoyl carbinol p-methoxybenzoyl carbinol * relative to benzoyl carbinol	+* -*	93
1960	Malinowski, Kehl, Tyrlik	glycolaldehyde-- PbO glyceraldehyde - PbO acetylacetone - PbO ethyl acetoacetate - PbO	0 0 0 0	94
1966	Balezin	sugar-like molecules	+	117
1966	Orestov	enediols	+	118
1969	Nakai, Sato, Tsujigado	D-fructose L-sorbose D-glucose D-mannose D-galactose D-arabinose L-arabinose	+ + + + + + +	128

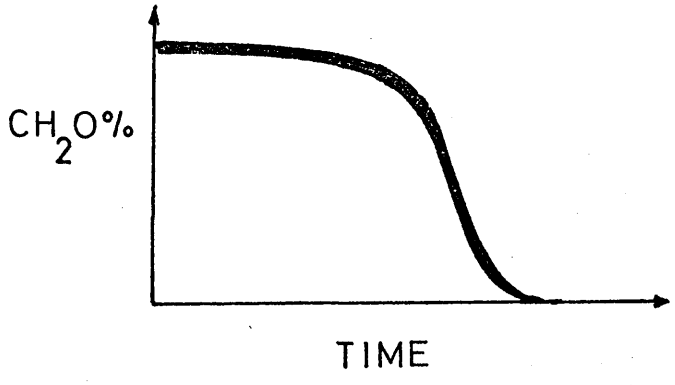
TABLE IV cont.

YEAR	INVESTIGATOR(S)	ACCELERATOR(S)	EFFECT	REFERENCE
1970	Krylov, Sinyak, Uspenskaya, Shul'gina	glucose	+	137

FORMOSE REACTION ACCELERATORS

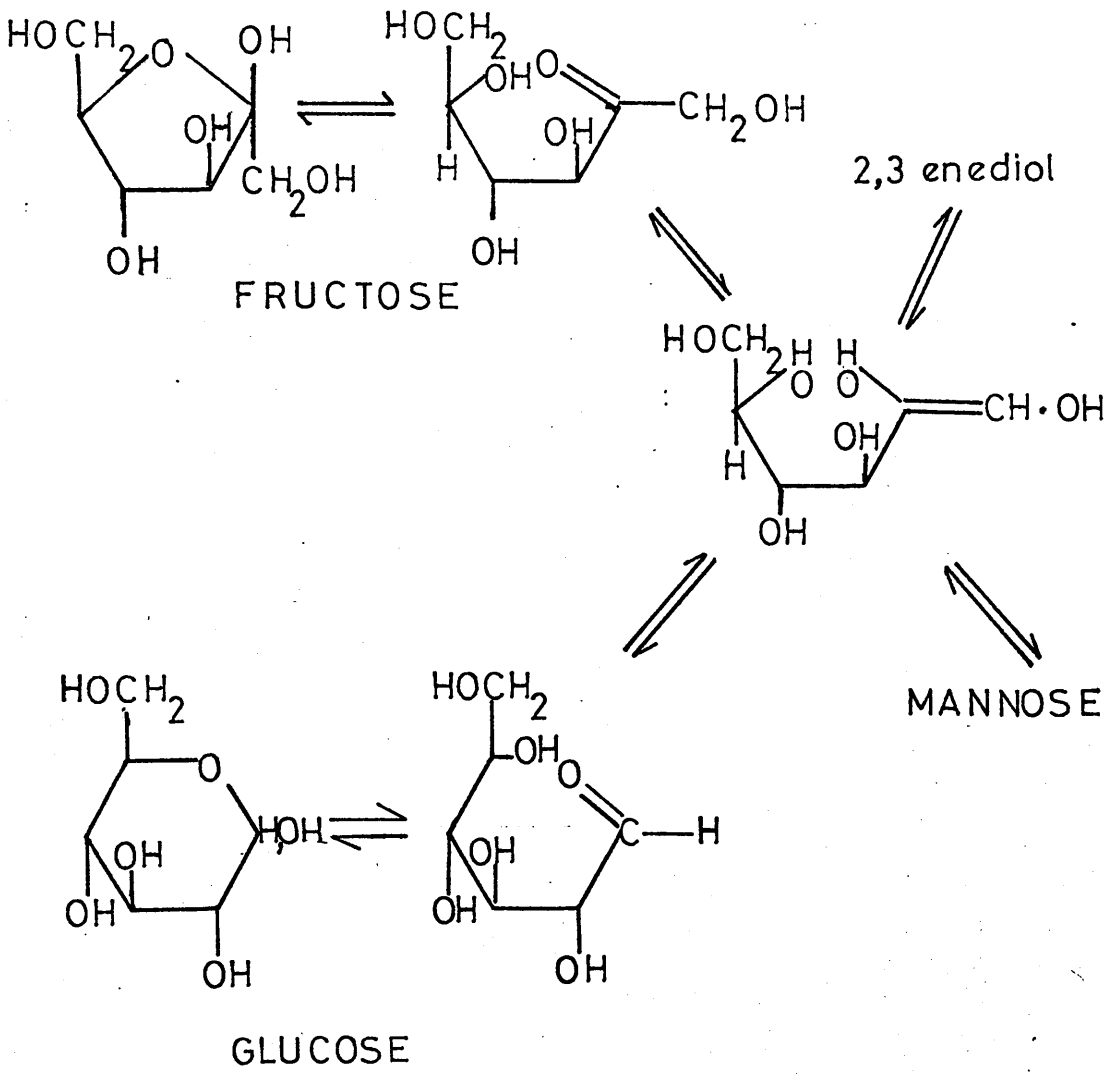
General Considerations.

If the colour of the solution is watched during a formose no change is observed for a time and then suddenly the solution starts to become brown. The brown colour, which is due to the caramelisation of the sugars, gives an indication, to a first approximation at least, of when sugars have been formed. If the formaldehyde concentration is monitored then a curve similar to that below is obtained.



A lag period or induction period is first experienced, followed by a phase during which the formaldehyde is rapidly consumed. The shape of the curve is similar to what we might have predicted knowing only the colour changes and the odour of the solution.

The lag period is thought to be due to the difficulty of the initial formation of glycolaldehyde, and the second, autocatalytic, stage due to the subsequent aldol condensations of glycolaldehyde, formaldehyde and their products, as we shall see shortly. It is not surprising to find that the addition of a small amount of sugar at the very start of the formose reaction alters the shape of the curve. By dispensing with the need for the initial formation of glycolaldehyde, the aldol condensations may start as soon as the additional sugar has been made available, with a consequent reduction in the induction period.


HOCH2
O
OH
OH
CH2OH
OH
FRUCTOSE
HOCH2
OH
O
CH2OH
H
OH
OH
2,3 enediol
HOCH2
H
H
O
O
CH·OH
OH
H
OH
HOCH2
O
OH
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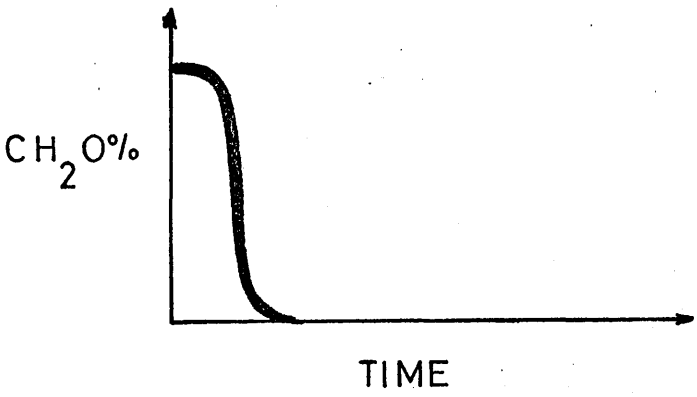
OH
HOCH2
OH
O
C—H
OH
OH
OH
MANNOSE
GLUCOSE



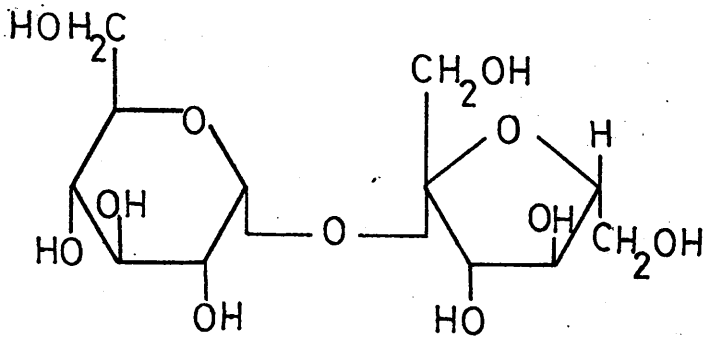
The curve takes on the new shape above.

Compounds possessing the ability to reduce or eliminate the lag period are called "accelerators". Compounds which prolong the period are "inhibitors".

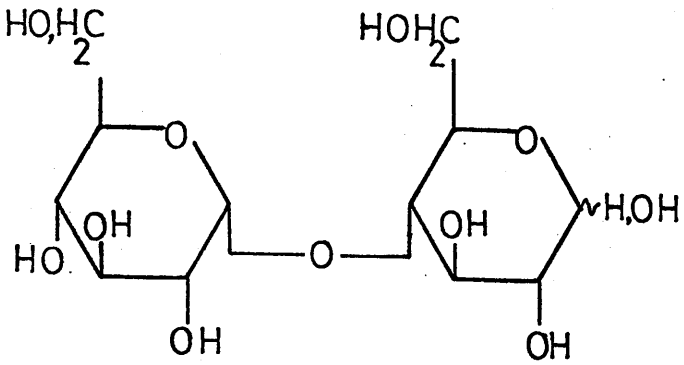
Table IV lists the known accelerators of the formose reaction and it can be seen that they are not all sugars. An investigation into the nature of the group that was responsible for the acceleration was undertaken by Kuzin in 1935.

The Active Group.

Kuzin decided that he would take a known formose accelerator and classify the various groups that were present in it, select model compounds on the basis of that classification and find whether they were able to accelerate the reaction. He chose fructose as his known accelerator, and listed the polyhydric alcohol group, the cyclic form with a hemiacetal hydroxyl, the keto group, the aldehydo group (from glucose and mannose), the enol and the ene-1,2-diol as potential accelerator groups.[48] Each model compound was studied in the lime-catalysed system.

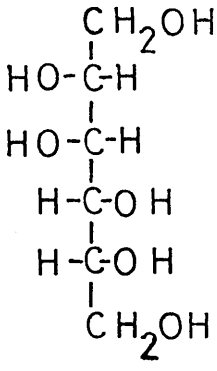


SUCROSE

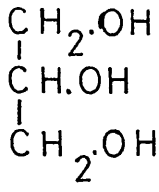


MALTOSE

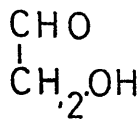
Mannitol and glycerol, polyalcohols, were devoid of any activity, so that group was discounted.



mannitol

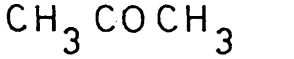


glycerol

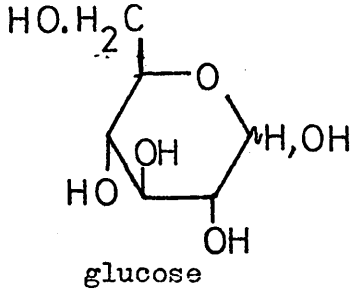


glycolaldehyde

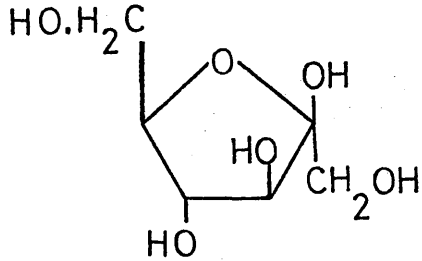
The amylene and butylene oxide forms were also discarded because an acceleration was found with glycolaldehyde (which cannot form these rings), and no effect was obtained with sucrose (which possesses both of them).



acetone



glucose

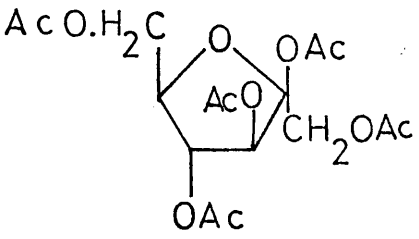


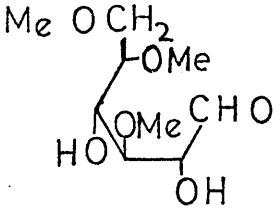
fructose

Acetone did not reduce the lag period, making the participation of the keto-group unlikely. The enol was also discounted on the basis of the same observation. The participation of the free aldehyde was thought unlikely, as a similar acceleration was experienced with glucose, fructose and maltose. A common factor was suggested, however, perhaps the ene-1,2-diol. The participation of this tautomer is supported on the basis of the earlier observations as well.

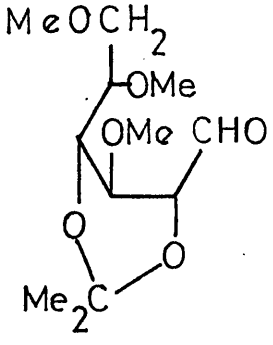
In order to establish the validity of this hypothesis a series of "blocked" compounds was selected by Kuzin.

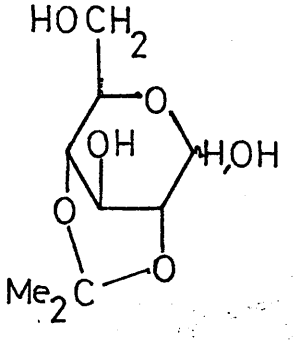
Acetylation of all the hydroxyls in fructose removed the ability to accelerate the reaction. In both penta-O-acetylfructose and sucrose the possibility of ene-1,2-diol formation is removed.





If cyclisation is prohibited and enolisation* allowed (in 3,5,6-tri O methylglucose) a more marked effect is experienced than with glucose alone. If both cyclisation and enolisation* are prevented (in 3,5,6-tri-O-methyl-; 2,4 – O-isopropylideneglucose), or, if enolisation* only is blocked (in 2,4 – O-isopropylideneglucose), there is no acceleration.

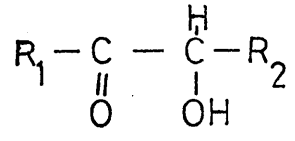


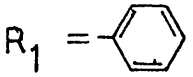


(* Enolisation resulting in <u>two</u> hydroxyls on the double bond.)

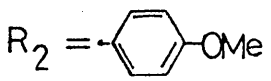
On the basis of these observations Kuzin concluded that the ene-1,2-diol was responsible for the observed accelerations.

The next question that he asked was whether compounds other than sugars, possessing the enediol group could accelerate the reaction. Benzoin, anisoin and acetoin were active.

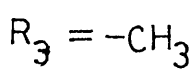




benzoin

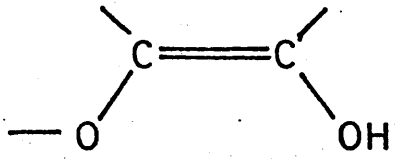


anisoin



acetoin

KUZIN'S MINIMUM REQUIREMENT FOR AN ENEDIOL ACCELERATOR



Is the double bond with only one hydroxyl active? The earlier result with acetone suggested that it was not, but confirmation was sought. As expected, ethyl acetoacetate, which can form an enol more readily than acetone because of conjugation to the carbonyl, does not alter the induction period. Ethoxyacetaldehyde, the ethyl ether of glycolaldehyde, does however cause an acceleration. Kuzin conc luded that the minimum requirement for an accelerating group was a double bond with two hydroxyl groups, one of which may be blocked.

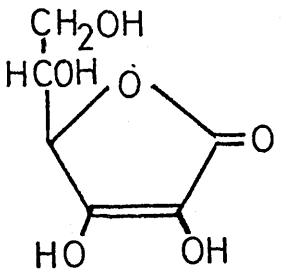
No attempts have been made to find whether the doubly-blocked enediol can be used, although it would seem unlikely that it would be so, because the enediol is usually considered to be a formaldehyde receptor through aldol condensation. (Other types of involvement of the enediol will be referred to later.)

$CH_3.CO.CH_2.COOEt$ ethyl acetoacetate

$EtO.CH_2.CHO$ ethoxyacetaldehyde (ethyl ether of glycolaldehyde)

Other accelerators.

The possibility of the formose reaction in plants had wide support during the earlier part of this century, so much significance was attached to the finding that ascorbic acid, vitamin C, could accelerate the formose reaction. We can see that the active enediol is well represented in this molecule.[52,55,57]

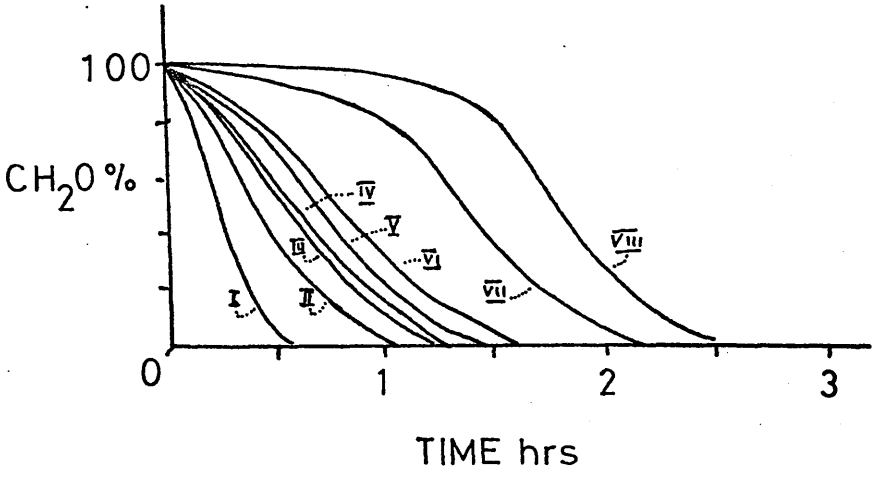


L-ascorbic acid

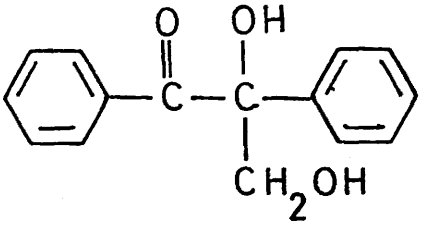
α-amino ketones reduce the induction period.[88,89]

THE EFFECT OF DIFFERENT ACCELERATORS ON THE RATE OF FORMALDEHYDE UPTAKE

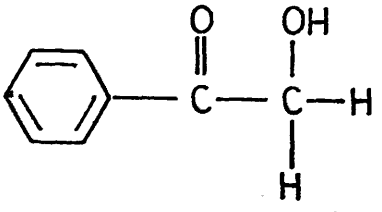
(after Langenbeck[75])



I benzoyl carbinol
II naphthoyl carbinol
III acetol (monohydroxyacetone)
IV 1,3-dihydroxyacetone
V glyceraldehyde
VI acenaphthoyl carbinol
VII fructose
VIII glucose



α-HYDROXYMETHYLBENZOIN



BENZOYL CARBINOL

A number of benzoin-derived accelerators have been found in addition to those that were mentioned before. The addition of a p-methoxy group to the ring lessens benzoin's effect, while a ring deactivating group (m-, p-cyano) increases it.[93] A A complex between benzoin and formaldehyde has been isolated.[70] and is a better accelerator than benzoin alone. After its use benzoin can be recovered from the formose - which is sweet-tasting! Removal of one of the phenyl groups from benzoin leads to benzoyl carbinol, another accelerator: naphthoyl and acenaphthoyl derivatives have a similar effect.

Although acetone itself does not change the length of the delay in the reaction, the monohydroxy- and 1,3-dihydroxy-derivatives do, both being efficient promoters.[48,49,72]

Needless to say, formose produces the expected result.[68] Most of the sugars that have been identified in formose have, at one time or another, been tested for their effect on the curve of formaldehyde concentration and, indeed, all accelerate the reaction, but to different extents. Only one paper appears in the literature where glycolaldehyde and glyceraldehyde were found to be devoid of activity: the conditions used in these experiments may not have been favorable for the formose reaction.[94]

Usually a small amount of accelerator is added, less than 10%, but if a large quantity of glucose is added an inhibition is found.[82] Sugars do not accelerate the reaction in proportion to their concentration.[79]

As expected the reaction rate increases with temperature,[118] and a value of the Arrhenius activation energy for the lime-catalysed reaction has been given as 11.3 kilocalories per mole.

The effect of different accelerators on the formaldehyde uptake is illustrated on the opposing page.

Inhibitors.

Alcohols may have an inhibiting effect on the formose reaction, although some cause an acceleration.[47,82] (Alcohols that are common organic solvents are being referred to here, not compounds like mannitol.) Effects are much less marked than with enediol compounds. and may be due to the solvation of the formaldehyde by the alcohols, as we shall see later.

Acid prolongs the time of the reaction,[43] and aromatic compounds such as p-nitrotoluene have a powerful inhibitory effect.[67,70] The aromatic compounds may destroy the sugars as they are formed.[70]

There are more formose reaction accelerators than inhibitors.

FORMOSE REACTION PRODUCTS

The Types of Assay.

Formose may be assayed in various ways, depending on the requirements. Often, in kinetic experiments where the detailed composition of the formose is not needed, the appearance of a brown colour accompanied by the release of caramel odour is sufficient indication that the formose reaction has occurred. In such cases the assignment is "sugar" or "no sugar".

The next level of assay gives information as to the amount of sugar produced. Estimates may be made of total sugar,[119] reducing sugar,[135] or fermentable sugar.[128] A further refinement leads to the individual families of sugar - aldose and ketose, and triose, tetrose, etc.. Paper chromatography is extremely useful for this and has been applied almost routinely in recent years.[83,90,95,96,111,113,114,115,119,120,121,124,128,130]

Paper chromatography is also suitable for the identification of individual sugars in formose, a further refinement in the method of analysis. Surprisingly gas-liquid chromatography has only been used once.[133] Further developments may be expected in its application as it provides a way of quantitatively measuring the amount of individual sugars. Thin layer and ion-exchange chromatography have not been often used.[116]

^{14}C tracers have been incorporated in formaldehyde at the start of the reaction to enable a quantitative estimate of triose. tetrose, etc., to be made.[119]

TABLE V. FORMOSE REACTION PRODUCTS

Abbreviations.

A number of abbreviations have been introduced in order to simplify the Table. The abbreviations come into two categories; those which indicate the number of carbon atoms in the sugar, and occasionally whether the sugar is an aldose (A) or 2-ketose (K), and those which are abbreviations for specific sugars. Thus C_{3A} and and C_{6K} represent aldotriose and ketohexose respectively. The specific sugars are abbreviated as follows:

G2	glycolaldehyde	All	allose
G3	glyceraldehyde	Alt	altrose
DHA	1,3-dihydroxyacetone	Glu	glucose
E	erythrose	Man	mannose
T	threose	Gul	gulose
Eu	erythulose	Id	idose
Rib	ribose	Gal	galactose
Arab	arabinose	Tal	talose
Xyl	xylose	Psi	psicose
Lyx	lyxose	Fru	fructose
Ru	ribulose	Sor	sorbose
Xu	xylulose	Tag	tagatose
DK	dendroketose		

Note.

Although identifications of various sugars have been made, they do not include the stereoisomers: this is understandable as no asymmetric catalysts have been used. But optically active accelerators have sometimes been added to start the reactions: whether this causes a bias of the products has not been investigated. Only in exceptional cases will D- or L- forms be mentioned, in accordance with practice in formose reaction reports.

The term "ketose" will be used to indicate a 2-ketose: 3-ketoses will be always prefixed.

TABLE V FORMOSE REACTION PRODUCTS

YEAR	INVESTIGATOR(S)	CONDITIONS	PRODUCTS	REFERENCE
1861-1908	Various		methylenitan, formose, methose, etc.	2,10-26
1909	Loeb	Zn, $ZnCO_3$, KOH	β-acrose	28,29
1919	Ewart	NaOH + salts of Ba, Sr	C_5, C_6	36
1924	Schmalfuss Kalle	MgO	DHA, C_{5-7}, ketose (no aldose)	37
1924	Kuster, Schoder	$MgSO_4$, $CaCO_3$, $CaHPO_4$, $Pb(OH)_2$	α-, β-acrose	38
1927	Schmalfuss, Congehl	MgO	DHA, C_5, C_6, Glu (no G2, G3)	39,43
1928	Vogel	MgO, PbO, $Ca(OH)_2$	d-acrose, "formose", C_5	41
1931	Karrer, Krauss	CaO	C_5, C_6, ?C_7	44
1938	Kuzin	$Ca(OH)_2$	G2, G3, C_5, C_6	58
1938	Prudhomme	Group II oxide + vis. & u.v. light	up to C_6	60
1939	Taylor, et al.	oxides and hydroxides of Mg, Ca, Sr, Ba, Sn, Pb	C_2, C_3, C_4	61
1940	Handford, Schreiber	Group II,IV compound + enediol	C_2, C_3, C_4, and higher, hydroxyaldehydes and ketones	62

TABLE V cont.

YEAR	INVESTIGATOR(S)	CONDITIONS	PRODUCTS	REFERENCE
1942	Handford,	Group IV compound enediol	hydroxyaldehydes, -ketones	64
1942	Lorand	Mg, Ca, Sr, Ba, Sn, Pb oxide hydroxide, carbonate		65,66
1948	Langenbeck	Pb, PbO	G2 ?	74
1961	Hough, Jones	$Ca(OH)_2$	C_5, C_{6A}, C_{6K}	81
1953	Mariani, Torraca	PbO	Rib, Arab, Xyl, Lyx, Ru, Xu, Glu, Man, Gal, Fru, Sor, and others	83
1954	Langenbeck	$Pb(OH)_2$	G2	85
1957	Balezin Surikina	$Ca(OH)_2$	Alt, ketose	90
1959	Binko, Kolar	Mg	G3, DHA	92
1960	Mayer, Jaschke	CaO*, 50°, 6h. (3%)*	G2, DHA, C_4, Rib, Arab, Xyl, Ru, Glu, Man, Gal Fru, Sor	95,96
		CaO*, 40°, 40min (30%)*	G2, DHA, Rib, Arab, Xyl Ru, Gal, Fru, C_4	
		CaO*, 40°, 5min (15%)*	G2, DHA, C_4, Xyl, Ru, Fru	
		CaO*, 40°, 20min (15%)*	Xyl, Ru, Fru	

TABLE V cont.

YEAR	INVESTIGATOR(S)	CONDITIONS	PRODUCTS	REFERENCE
1961	Pfeil Ruckert	$Ca(OH)_2$	G2, G3, DHA followed by C_4, C_{4K}, C_{5K}, Rib, Arab, Xyl or Lyx, Glu, Man, Gal, Fru, Sor, Dk, Rh	97
1964	Akerlof	alkali	C_5, C_6 and other sugars	110
1964	Orestov	CaO	Glu	111
1965	Runge, Mayer	pyridine, picolines, collidines	C_3, C_4, C_{5A}	114
1965	Ruckert, Pfeil Scharf	$Ca(OH)_2$	C_{3A}, C_{3K}, C_{4A}, C_{4K}, C_{5A}, C_{5K}, C_{6A}, C_{6K}, C_{7K}, Dk	115
1965	Ito	CaO	Fru	116
1967	Gabel Ponnamperuma	Al_2O_3 kaolinite, illite	G2, C_3, C_4, C_5, C_6 C_3, C_4, C_5, C_6	119
1967	Reid, Orgel	$CaCO_3$, carbonate-apatite	C_5, C_6	120
1967	Verbrugge	alkali	C_{5A}(Rib, Xyl), C_{6K}(Fru, Sor), Glu, Man, Gal	121
1968	Weiss, Shapira	$Ca(OH)_2$	C_3, C_4, C_5, C_6, $>C_6$	124
1968	Reisz	zeolites + γ-irradiation	C_9, C_{10}, or unlimited	125
1970	Weiss, Shapira	$Ca(OH)_2$	C_2, C_3, C_4, C_5, C_6, $> C_6$	133

TABLE V cont.

YEAR	INVESTIGATOR(S)	CONDITIONS	PRODUCTS	REFERENCE
1970	Mizuno, Shiomi, Mori, Nakatsujii	Group I, II hydroxides: organic bases (see Table I)	C_3, C_4, C_{5A}, C_{5K}, C_{6A}, C_{6K}, C_{7K}	135

The Constitution of Formose.

The organic components of the formose liquor may be classified as follows:[30]

i formic acid and methanol;

ii sugar and caramel.

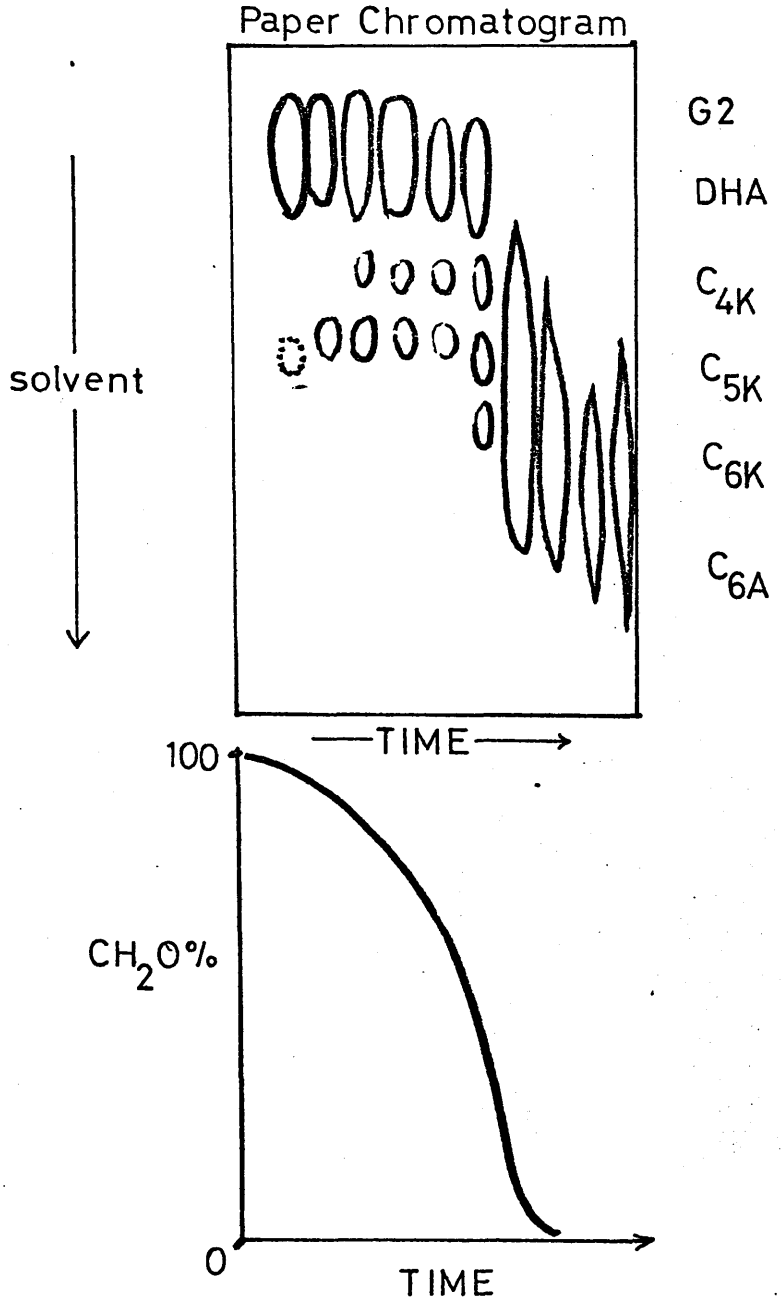
The first molecules arise from the Cannizzaro reaction, which is the major reaction before the end of the lag period. A small quantity of formic acid may be formed by atmospheric oxidation of formaldehyde, and by a cross-Cannizzaro reaction of CH_2O and sugar.

The compounds in the second category are the true formose products - the sugars that are formed from formaldehyde, and their decomposition products. The products of a sugar nature that have been found in formose have been recorded in Table V. The composition of caramel is complex and will not be dealt with at the moment, but an indication of some of the compounds that have been found in caramel is given in Appendix III.

Formose sugars are, in general, straight-chain aldoses and ketoses containing from two to seven carbon atoms. Polysaccharides are not formed, and branched-chain sugars, such as dendroketose,[115] have not often been identified. In view of the large number of potential condensations between lower sugars, it is rather surprising that more branched sugars have not been found. They may constitute part of the unresolved material in formose.[46,83,133,137] The reason for the maximum size of sugar containing only about six carbon atoms is thought to be due to the thermodynamic advantage that cyclisation offers.

Formose is a mixture, and in that mixture both the quantity and the type of sugar may vary. The mixture is not static, for pentoses have been found to be in a maximal amount after 12 hours and hexoses 24 hours.[120] Prolonged refluxing leads to the destruction of the sugars.[120] Sometimes single sugars have been isolated in large yield e.g. glycolaldehyde[85] or

VARIATION IN COMPOSITION OF FORMOSE
WITH UPTAKE OF FORMALDEHYDE
(after Pfeil and Ruckert[97])



fructose.[116] Sometimes all the aldoses and ketoses with between two and six carbon atoms have been found.[115]

As we would expect, lower sugars are formed before the higher ones: this is well illustrated by Pfeil and Ruckert's kinetic chromatogram (opposite). The time for formation of hexoses may be very soon after the start of the reaction - glucose, fructose and mannose have been isolated after one minute.[111]

The prediction of the composition of formose is not possible 'ab initio', but mixtures of predominantly triose, say, can be achieved by manipulating the time, pH, etc. and other reaction variables. Such practices are often employed when formose is being produced industrially.[58,62,65,74,96,124] Glyceraldehyde is said to have a directive effect on the products.[130] (Patents covering the industrial preparation of formose are summarised in Appendix V.)

THE FORMATION OF FORMOSE IN THE ABSENCE OF ORGANIC AND INORGANIC CATALYSTS

The formose reaction may occur in the absence of the conventional catalysts. Sugars are formed when formaldehyde is subjected to ultraviolet rays,[31,32,36,113,161,176,184,199,202] γ-rays,[113] and Tesla oscillations (electrical discharge).[80] Glycolaldehyde,[31,32] sugars up to hexose,[60] ribose and 2-deoxyribose[113] have been found in the product.

The breaking of the "pentose - hexose" barrier has been achieved by the combination of γ-rays and a suitable substrate. The possibility of formaldehyde molecules aligning themselves on a clay was suggested by Cairns Smith.[257] Recently it was shown that zeolites are suitable for this — C_9 to C_{10} polyhydroxy compounds were formed after γ-irradiation of A and X molecular sieves. The carbon chain length was of unlimited size when substrates with channel-like holes were used.[125]

So far in this review we have listed the various catalysts, accelerators and products of the formose reaction. It has already been suggested that aldol condensations occur among the sugars (they are listed in Appendix I), and that the observed lag time is due to the initial difficulty in forming glycolaldehyde. It is now time to discuss the kinetics and mechanism of the reaction in greater detail. In doing so, we shall review the various reaction intermediates that have been suggested, and see whether there are any reactions analogous to the initial reaction in the literature.

FORMALDEHYDE SALTS *

METAL	APPROXIMATE COMPOSITION	METAL: CARBON RATIO
Calcium	$Ca(O.CH_2.OH)_2$	1:2
Strontium	$CH_2.(O.Sr.O.CH_2.OH)_2 \cdot 7H_2O$	2:3
Copper	$Cu.(O.CH_2.O.Cu.O.CH_2OH)_2 \cdot 2H_2O$	3:4
Zinc	$CH_2.(O.Zn.OH)_2 \cdot 2H_2O$	2:1
Cadmium	$CH_2\ (O.Cd.O.CH_2)_2.O.Cd.OH_{\ 2} \cdot 9H_2O$	6:5
Lead	$CH_2\ (O.Pb.O.CH_2)_2.O.Pb.OH_{\ 2} \cdot 2H_2O$	6:5
Barium	not given	
Magnesium	not given	

* After Franzen.[33,34]

FORMOSE REACTION INTERMEDIATES

A number of compounds have been isolated, which may be actual formose intermediates, or models for them. We shall see that they may be conveniently classified into combinations of formaldehyde, the catalyst and the accelerator.

I Complexes of the Catalyst and Formaldehyde.

a. Formaldehyde hydrate.

Formaldehyde salts of several metals are known: they are summarised on the facing page. With the exception of the cupric salt, which is green, they are all white solids, smelling of formaldehyde and completely soluble in water. Their compositions vary from a metal-to-carbon ratio of 1 : 2 to 2 : 1. On standing the strontium and barium salts decompose to a brown liquid with the odour of caramel.

Unsuccessful attempts were made to isolate the sodium, nickel, aluminium and ferric salts,[33,34] but in solution an association between formaldehyde and sodium may exist.[141] Staudinger suggested that these solids may not be definite compounds, but simply mixtures of alkaline earth hydroxides and formaldehyde polymers.[45]

The lead oxide-formaldehyde complex is an active catalyst for the condensation.[94] The existence of a lime complex (Euler)[25] has not been confirmed by Kuzin.[55]

The existence of such species during the formose reaction has been suggested a few times,[71,117,124,133] and they are a necessary part of the Gabel and Ponnamperuma mechanism.[119]

b. Anhydrous formaldehyde.

As an analogy to this we may use the known adsorption of acetaldehyde on alumina, where the carbonyl oxygen is bonded to the surface.[103]

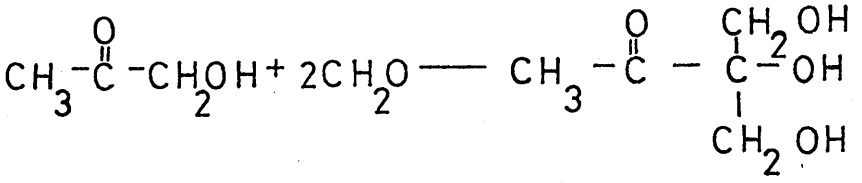
II Complexes between the Catalyst and Accelerator.

Calcium fructosate[48] and calcium glucosate[48,131] are formose accelerators. PbO forms complexes with glycolaldehyde and glyceraldehyde, but these are said to be inactive. There may be some uncertainty attached to the conditions used here - they may not have been suitable for the formose reaction in the absence of the sugar promoters.[94] Complexes of sugars and alkali metals and the alkaline earths have been reviewed elsewhere.[142]

III Complexes between the Accelerator and Formaldehyde.

Glycolaldehyde is known to form glyceraldehyde in the presence of formaldehyde.[96] The other aldol condensations of sugars up to C_4 and formaldehyde are reviewed in Appendix I. Glucose and fructose react only slowly with formaldehyde.[133]

Bishydroxymethyl-acetol was made by Katzschmann by reacting acetol with formaldehyde. The two formaldehyde molecules add onto the same carbon atom[69], which he used as evidence for one of the intermediates in his mechanism.



Hydroxymethyl derivatives of non-sugar acyloins are known. The benzoin complex accelerates the formose reaction to a greater extent than benzoin alone.[50,63,70] A formaldehyde--ascorbic acid compound can be formed even in acid.[51]

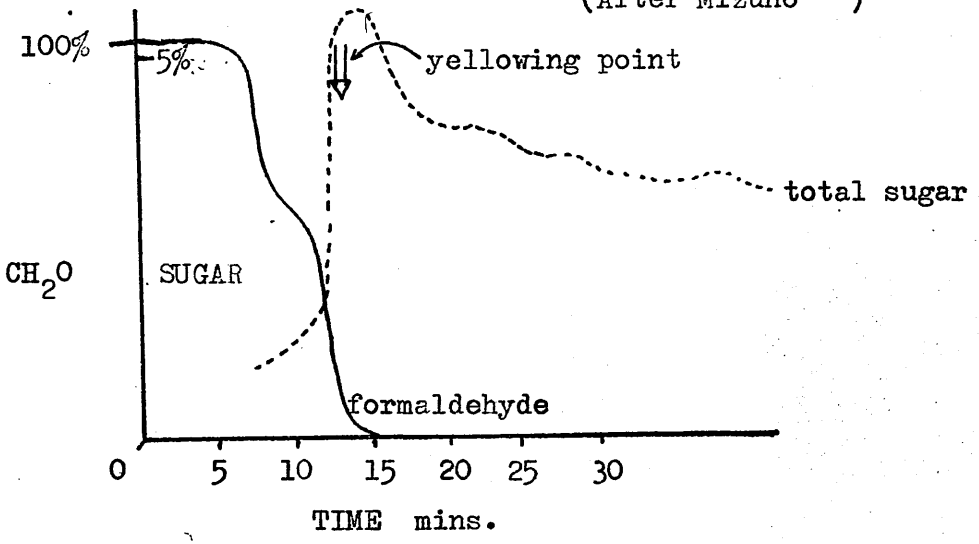
No adduct between benzoyl carbinol and formaldehyde has been mentioned in any of the references under review.

III Complexes of Catalyst, Formaldehyde and Accelerator.

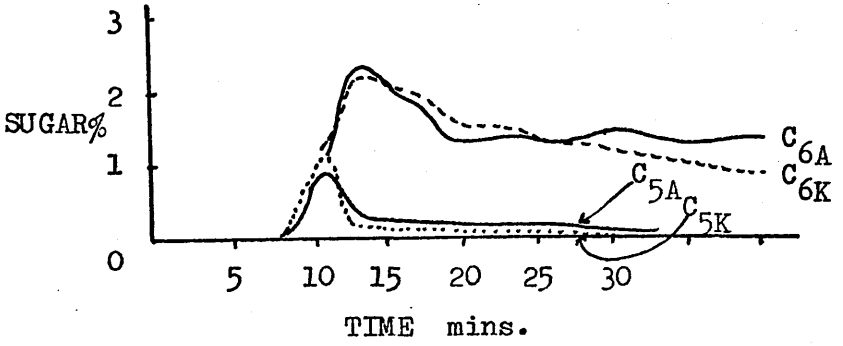
Calcium saccharates unite with formaldehyde to form unstable intermediates,[48,54] and the formaldehyde involved is a single carbon unit rather than a polymer.

COMBINED CURVES OF FORMALDEHYDE CONSUMPTION AND SUGAR FORMATION IN THE LIME - CATALYSED FORMOSE REACTION

(After Mizuno[135])



FORMATION OF ALDO- AND KETO-PENTOSE AND -HEXOSE DURING THE ABOVE REACTION



FORMOSE REACTION KINETICS

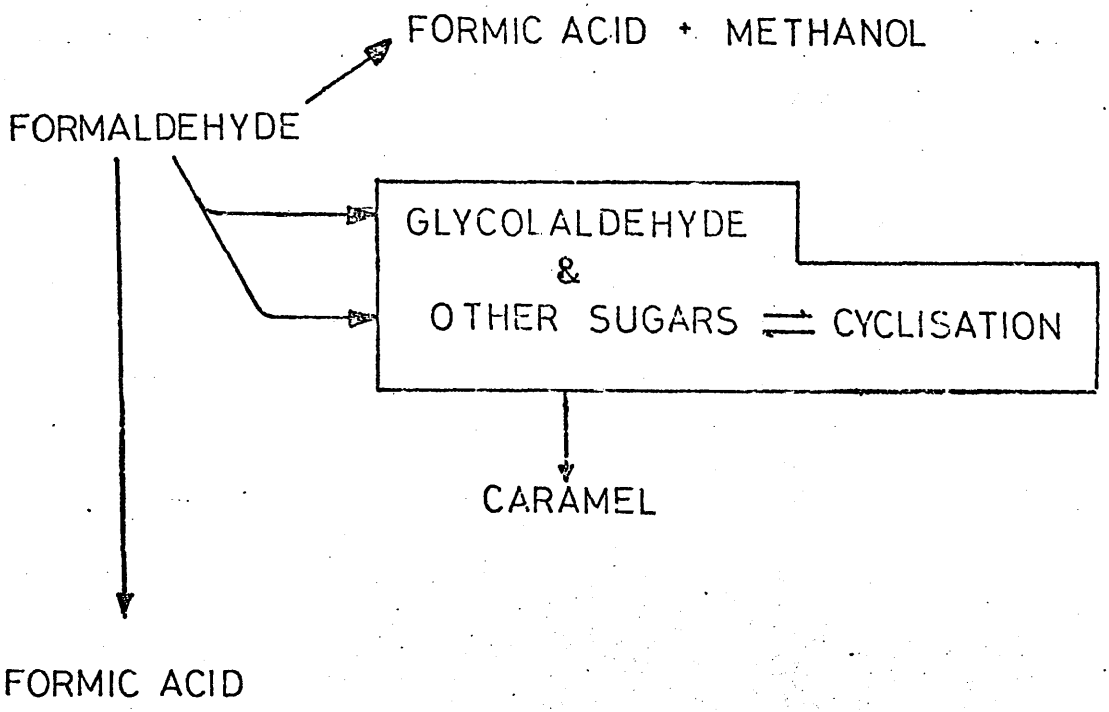
A variety of physical and chemical parameters may be used in following the kinetics of the reaction - changes in the volume of solution,[54,59,72] electrical conductivity,[71] thermal content,[118] high-frequency behaviour,[111] formaldehyde concentration,[82,93,128] sugar content,[135] and catalyst solubility.[71]

The same general characteristics have been found with all of these methods - a lag period and a rapid (autocatalytic) stage.

The most often measured variable is the formaldehyde concentration, for which only three references out of many have been given above. Occasionally the product has been sampled at the same time as the aldehyde estimation. Pfeil and Ruckert's kinetic chromatogram is one example (given earlier) and Mizuno's combined formaldehyde and sugar assay is another (facing). From this graph we can see that the yellowing time is when the formaldehyde concentration is almost zero. The inflection in the formaldehyde curve may be due to the occurrence of the cross-Cannizzaro reaction. The inflection is not found in other formaldehyde concentration curves.

Mizuno combined his total sugar measurement with a determination of aldohexose, etc. The maximum concentration of hexose comes after the maximum pentose value: the closeness of the aldose and ketose curves for either pentose or hexose suggests that isomerisation of the carbonyl group is very rapid. Prolonged refluxing reduces the sugar concentration, due to caramelisation. Mizuno prepared a similar curve for an organic catalyst, and the same characteristics were shown.

REACTIONS IN AND AROUND FORMOSE



Kinetic analyses of the reactions in the formose system are complicated by the large number of reactions that occur. The reactions involved are summarised on the opposite page. We should also remember that each compound will have its own rate constant for a particular reaction.

Shapira, Weiss and La Pierre had, however, some success in treating the homogeneous, lime-catalysed reaction mathematically. They derived the following expression on the basis of experimental measurements: it accounts for the production of two, three and four carbon sugars in their continuous stirred tank reactor.

$$\frac{d}{dt}\left[A_2 + A_3 + A_4\right] = 0.0582\left[A_1 + A_2 + A_3 + A_4\right]\left[A_2 + A_3 + A_4\right]\left[Ca(OH)_2\right]$$

where A_i is the concentration of sugar with i carbon atoms.

The expression is third order, involving the catalyst, formaldehyde and sugars. At intermediate conversion levels the formaldehyde reaction rate was proportional only to the lime concentration.[133]

More recent results suggest that a critical catalyst concentration may be required.[136]

The rate controlling step was found to be product decomplexing: the reaction took place betweeen complexed formaldehyde and also complexed intermediate. (A mechanism involving both species complexed on a common centre did not satisfy the experimental data.[133])

Depolymerisation of formaldehyde polymers was found to be at a sufficiently slow rate to be important in the overall rate, but was successfully incorporated in the kinetic expression.[136]

FORMOSE REACTION MECHANISMS

Introduction.

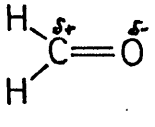
All mechanisms for the formose reaction in an aqueous environment invoke glycolaldehyde as the primary product. Before looking at these mechanisms in detail we should be sure that this molecule is formed. Glycolaldehyde is one of the sugars that is isolated in formose (Table V) and it is the sugar that one would expect to see first. The kinetic chromatogram of Pfeil and Ruckert shows that a compound with paper chromatographic characteristics like glycolaldehyde has been formed before the appearance of the higher sugars. We may be reasonably certain that glycolaldehyde is indeed the primary product of the formaldehyde condensation.

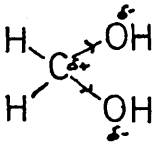
For the sake of clarity the two stages in the reaction will be called Stage I and Stage II: the former refers to the primary reaction, the initial formation of glycolaldehyde, and the latter to the second (aldol) stage where sugars are formed.

The Mechanisms.

STAGE I The Initial Formation of Glycolaldehyde.

The combination of two molecules of formaldehyde is formally the most difficult step because one formaldehyde carbon atom must become a nucleophile.* Normally this carbon atom is electrophilic because of the inductive effect of one oxygen atom in the carbonyl form of the molecule and two oxygen atoms in the hydrate.





* There is no evidence for a radical reaction in aqueous solution.

i Mechanisms involving unhydrated formaldehyde.

Two reaction schemes have been proposed for stage I, involving formaldehyde itself. The first of these, by Pfeil and Schroth,[82] was formulated in 1951.

a. The Pfeil and Schroth mechanism.

After a number of experiments, Pfeil and Schroth gave a list of reaction characteristics that have to be included in any theory.[82]

1 The Cannizzaro reaction accompanies the formaldehyde condensation.

2 Small amounts of glucose and alcohols (methanol, ethylene glycol, etc.) accelerate the reaction: large amounts retard it.

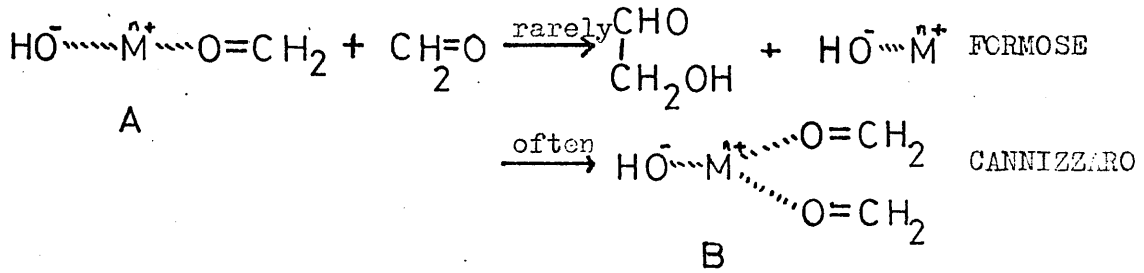
3 The various catalysts do not act in the order of their basic strengths (see opposite).

4 Salts of the same cation as the alkali accelerate the formose reaction: salts of cations whose alkalies are less efficient catalysts slow the reaction down.

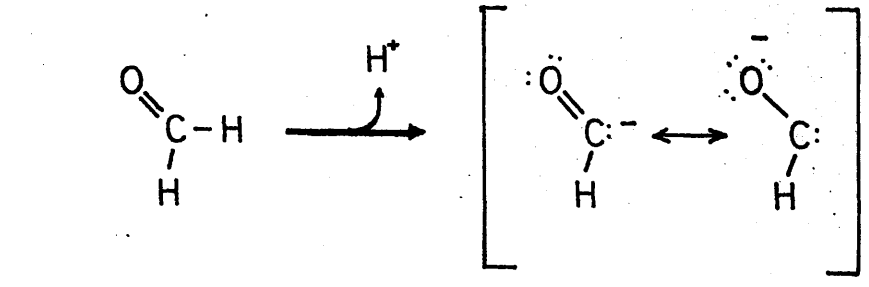
5 A high formaldehyde concentration and a high ratio of formaldehyde to catalyst favour the Cannizzaro reaction.

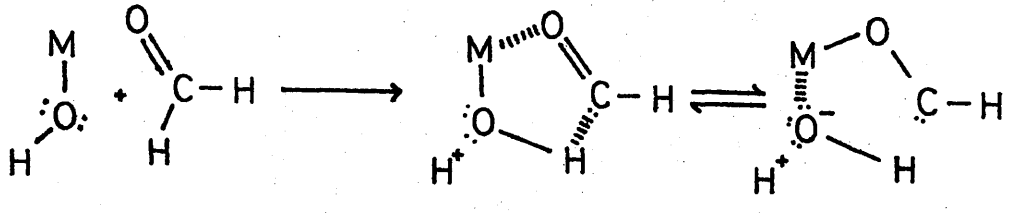
6 The velocity of the Cannizzaro reaction is proportional to the square of the formaldehyde concentration: the formose reaction has first order proportionality.

They proposed that the formation of glycolaldehyde initially resulted from the reaction of A with free formaldehyde. By coordination to the metal the acidity of the methylenic hydrogens is increased. The second molecule of formaldehyde may enter complex A to give the Cannizzaro complex, B.



THE WANZLICK MECHANISM





During the induction period the formation of B occurs much more often than the primary reaction. Slowly glycolaldehyde is formed, allowing the second stage to begin. (We shall look at Pfeil and Schroth's mechanism for the second stage shortly.)

This mechanism satisfies criteria 1, 3, 4, 5 and 6: alcohols added (2) in large amount cause hemiacetals of formaldehyde to be formed. These obviously cannot enter Pfeil and Schroth's complex so the effective concentration of formaldehyde is reduced with a reduction in the reaction rate.

b. The Wanzlick mechanism.[101]

Wanzlick outlined the way in which ionisation of formaldehyde on a metal centre might happen. The possibility of a carbene as the nucleophile was raised in this way. The mechanism (opposite) is similar to the first part of Pfeil and Schroth's.

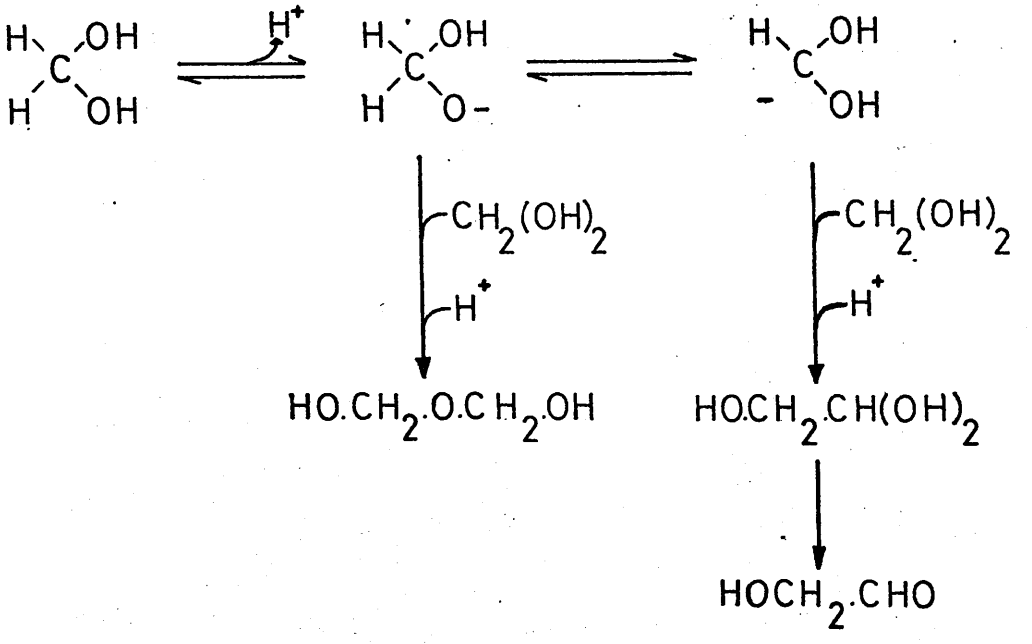
II Mechanisms involving formaldehyde hydrate.

Three rather similar mechanisms may be found in the literature for the first stage, involving dihydroxymethylene rather than formaldehyde. The more complete mechanisms will be discussed, that by Gault[84] will not be mentioned in detail.

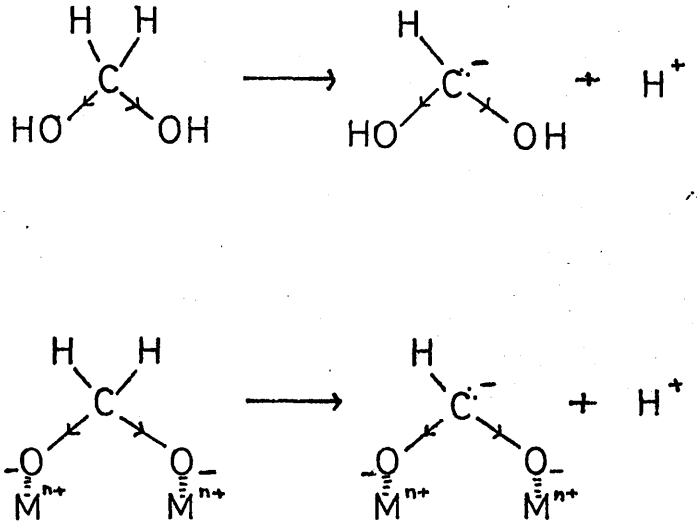
a. The Gabel and Ponnamperuma mechanism.

Both of the oxygens in dihydroxymethylene exert an inductive pull on the carbon atom: this in turn increases the acidity of the methylenic hydrogen atoms. On a cation-bearing surface coordination of formaldehyde hydrate, perhaps with salt formation, will occur. The effect of the cations increases the acidity of the methylenic hydrogens further, enabling carbanion formation at a lower pH. Reaction of this species with

THE RUNGE AND MAYER MECHANISM - Part I



formaldehyde, monomer or hydrate, gives glycolaldehyde.

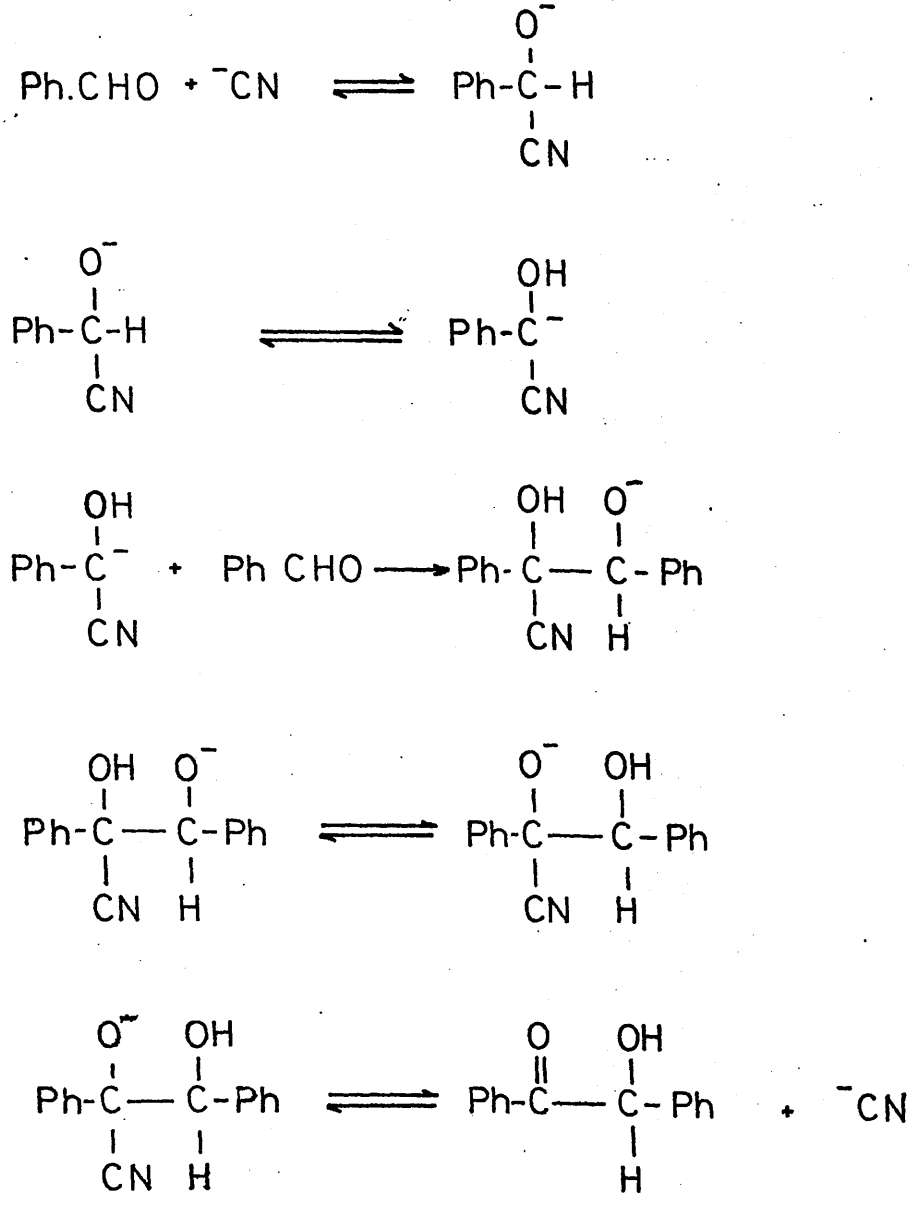


b. The Runge and Mayer mechanism.[102]

Ionisation of the hydroxylic hydrogen atoms would be expected to happen more easily than ionisation of the methylenic hydrogen atoms in formaldehyde hydrate. Runge and Mayer have used this distinction to show the difference between polymer and condensate formation (opposite). The difference lies only in the site of the nucleophile, oxygen or carbon. A proton shift from the carbon to the oxygen will lead to the carbanion.

We shall now digress slightly to look in the literature for known reactions which resemble the first stage of the formaldehyde condensation, in order to see whether the mechanisms involving hydrated or unhydrated formaldehyde are favoured. We do not have to look far, because the well known Benzoin Condensation gives us an example.

LAPWORTH'S MECHANISM FOR THE BENZOIN CONDENSATION



The Benzoin Condensation.

The formation of benzoin from benzaldehyde has been known for many years. It is a reaction for which the cyanide ion is a specific catalyst. Lapworth's mechanism (opposite) was accepted for many years although the experimental basis for it was weak.[146] Recently a close examination of the reaction was made, in general confirming the earlier mechanism.[145]

Inorganic cyanide is a specific catalyst for the reaction, and may be present as the sodium, potassium or tetrabutylammonium salt.[144,145] (The advantage of the latter cation is that it is more soluble in organic solvents than the former cations.) Cyanohydrin formation increases the acidity of the aldehydic hydrogen atom, enabling carbanion formation to occur more easily. Condensation of this ion with free benzaldehyde and carbanion formation compete as the rate determining steps.[145]

Ring substituents which increase the electron density on the aldehydic carbon, while not greatly affecting the cyanohydrin formation, do tend to reduce the probability of condensation: substituents which decrease the electron density reduce the reaction rate as well, because the carbanion can be stabilised.[143]

The similarity between the initial stage of the formose reaction and the benzoin condensation is close: both result in acyloins. From the above mechanism the hydrate mechanism of formaldehyde condensation might seem favoured. If this is so we should expect that cyanide would also increase the rate of the formose reaction.

Surprisingly, there is no mention of the addition of cyanide in the formose literature. Langenbeck[86] found that the accelerating ability of benzoyl carbinol was enhanced by cyanide, but we should exert a little caution in applying this to

the formose system, as benzaldehyde (coming from benzoyl carbinol by retro-benzoin condensation) may have introduced another pathway for the formation of glycolaldehyde.

STAGE II The Autocatalytic Reactions

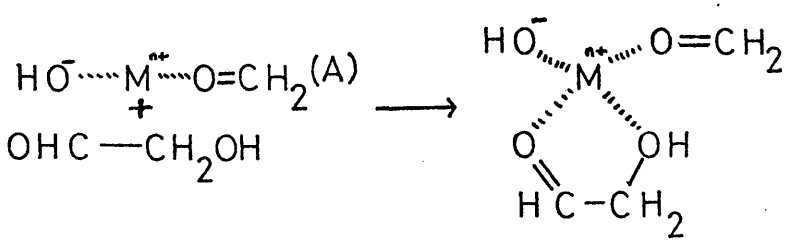
The second stage of the formose reaction is autocatalytic.[60,70,133] Two possibilities arise - either the primary reaction becomes self-catalysing, or a second (autocatalytic) pathway is provided, which is separate from the first. The latter way may be through aldol condensation.

The role of the enediol in these may be as a ligand to the cation of the catalyst, a passive involvement, or as an (active) receptor of formaldehyde through aldol condensation. We shall look at the passive role first of all.

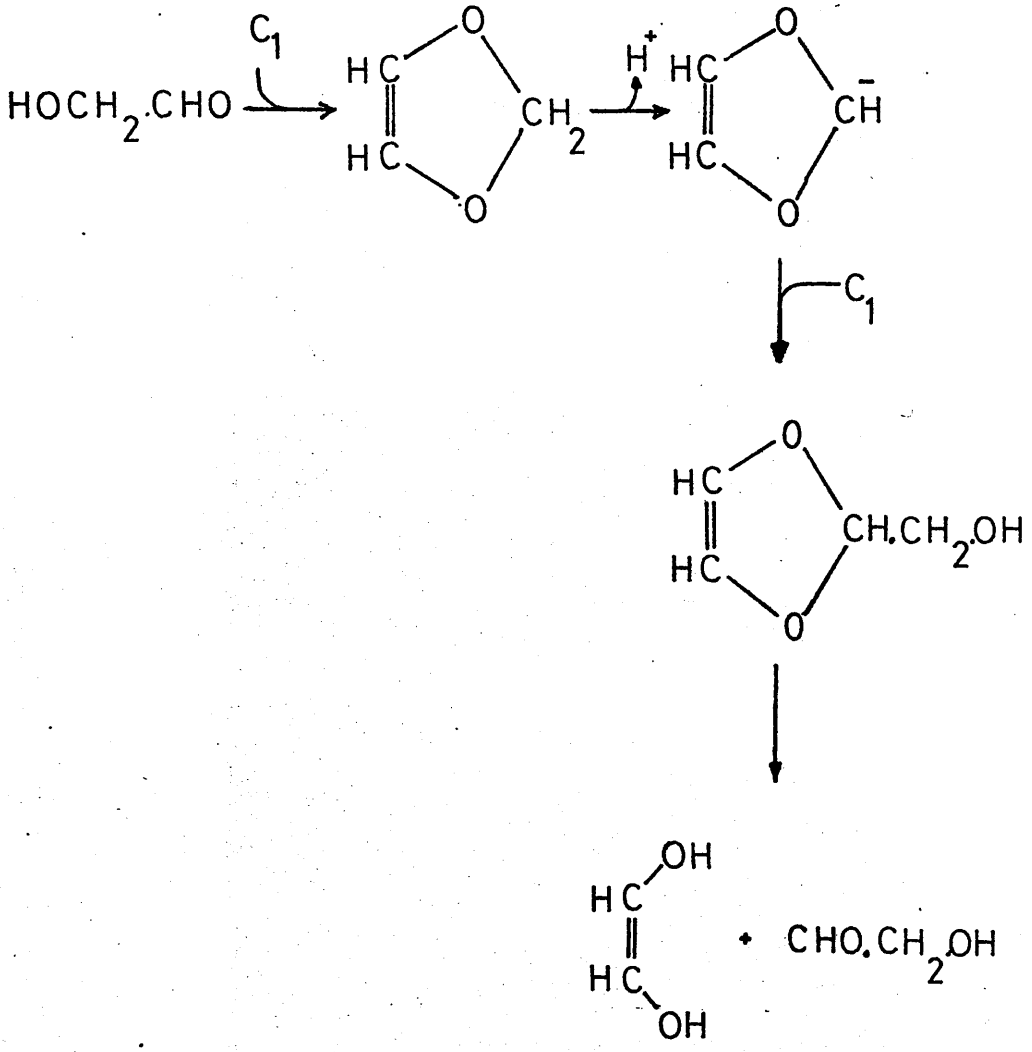
Acceleration of the Primary Reaction

a The Pfeil and Schroth mechanism.

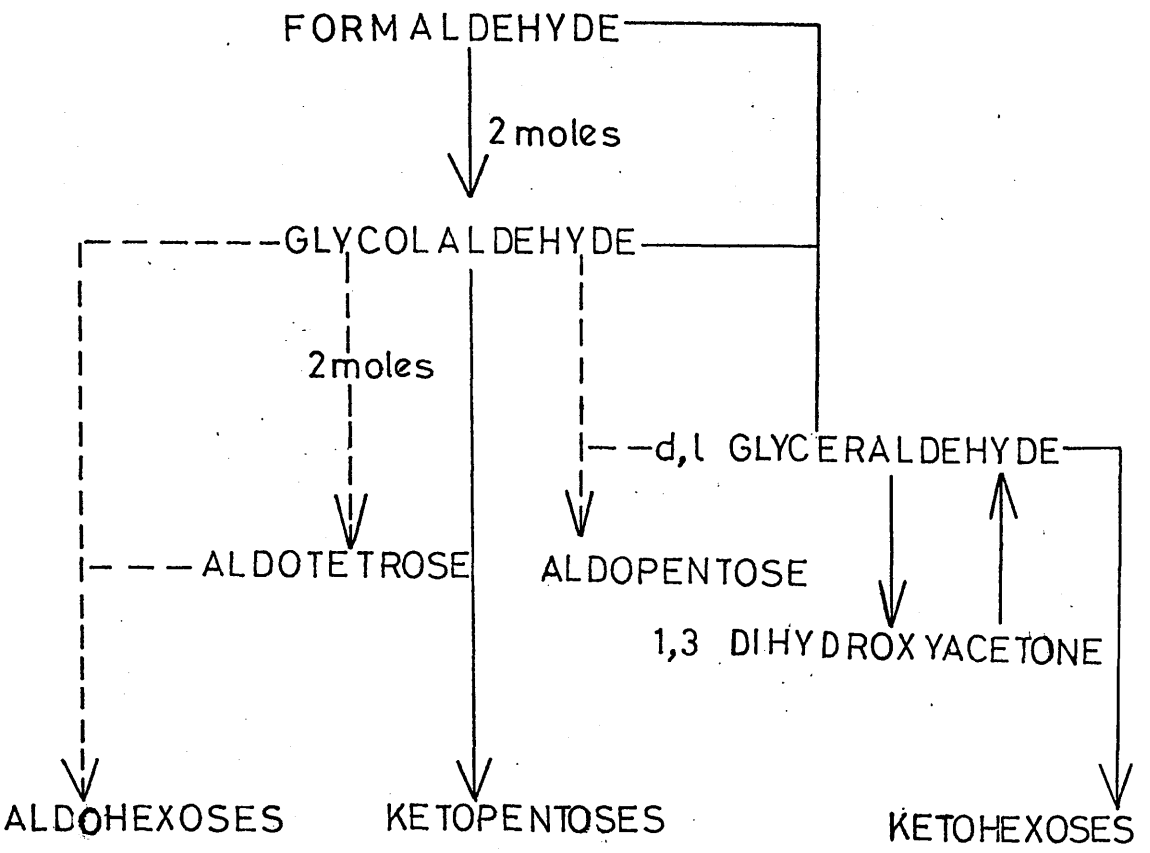
Once glycolaldehyde has been formed, it may enter the complex, A. By doing so it occupies two positions on the cation removing them from availability to formaldehyde. The formation of the Cannizzaro complex, B, is therefore hindered and the formation of glycolaldehyde through this <u>accelerated primary reaction</u> takes place.



THE RUNGE AND MAYER MECHANISM - Part II



ALDOL SCHEME - ORTHNER & GERISCH



b. The Runge and Mayer mechanism.

The possibility of formaldehyde becoming activated through the intermediacy of a cyclic species with the accelerator was suggested by Runge and Mayer (opposite). The involvement of this compound fails to explain why different metals should alter the rate of the reaction. Ethoxyacetaldehyde was found by Kuzin to accelerate the reaction;[49] it cannot form a five-membered ring with formaldehyde, making this mechanism rather unlikely. An alternative role for the ethoxyacetaldehyde is hard to see.

The Availability of an Alternative Pathway.

a. Aldol Condensations.

The most commonly accepted mechanism for the second stage of the formose reaction involves aldol condensations of formaldehyde, glycolaldehyde and other aldose and ketose sugars. Aldol condensations of sugars are known in their own right and have been summarised in Appendix I.

The first scheme relating some formose products through known aldol condensations was published by Orthner and Gerisch in 1933 (opposite).[46] All aldose and ketose sugars with from two to six carbon atoms have been accounted for, except ketotetrose. It is interesting to note that only one isomerisation has been used in this scheme and that there are no retro-aldol reactions (the aldol arrows are unidirectional).

Ruckert. Pfeil and Scharf constructed a table of the expected products of aldol condensation of the lower sugars.[115] In that we see that all the C_2 to C_6 aldoses and ketoses are present, except 1,3-dihydroxyacetone. A number of branched sugars can also be formed. This table is given in Appendix I.
The isomerisation of the carbonyl group in sugars and enolisation will

THE KATZSCHMANN MECHANISM (1944)

$$2CH_2O \xrightarrow{\text{slowly}} HO.CH_2.CHO$$

$$HO.CH_2CHO + CH_2O \longrightarrow HO.CH_2.CH(OH).CHO$$

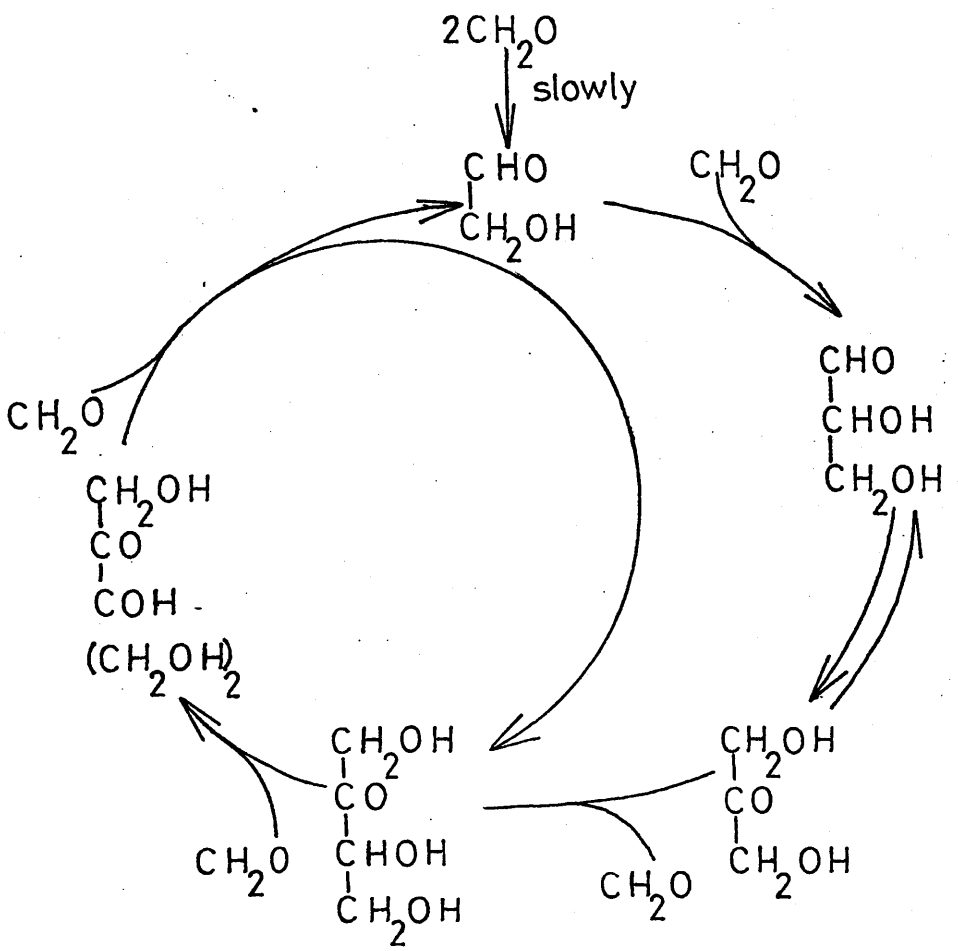
$$HO.CH_2.CH(OH).CHO \rightleftharpoons HO.CH_2.CO.CH_2OH$$

$$HO.CH_2.CO.CH_2OH + 2CH_2O \longrightarrow HO.CH_2.CO.C(OH)(CH_2OH)_2$$

$$HO.CH_2CO.C(OH)(CH_2OH)_2 + CH_2O \longrightarrow$$

$$HO.CH_2.CHO + HO.CH_2CO.CH(OH)CH_2OH$$

(quickly)



be mentioned in Appendix II.

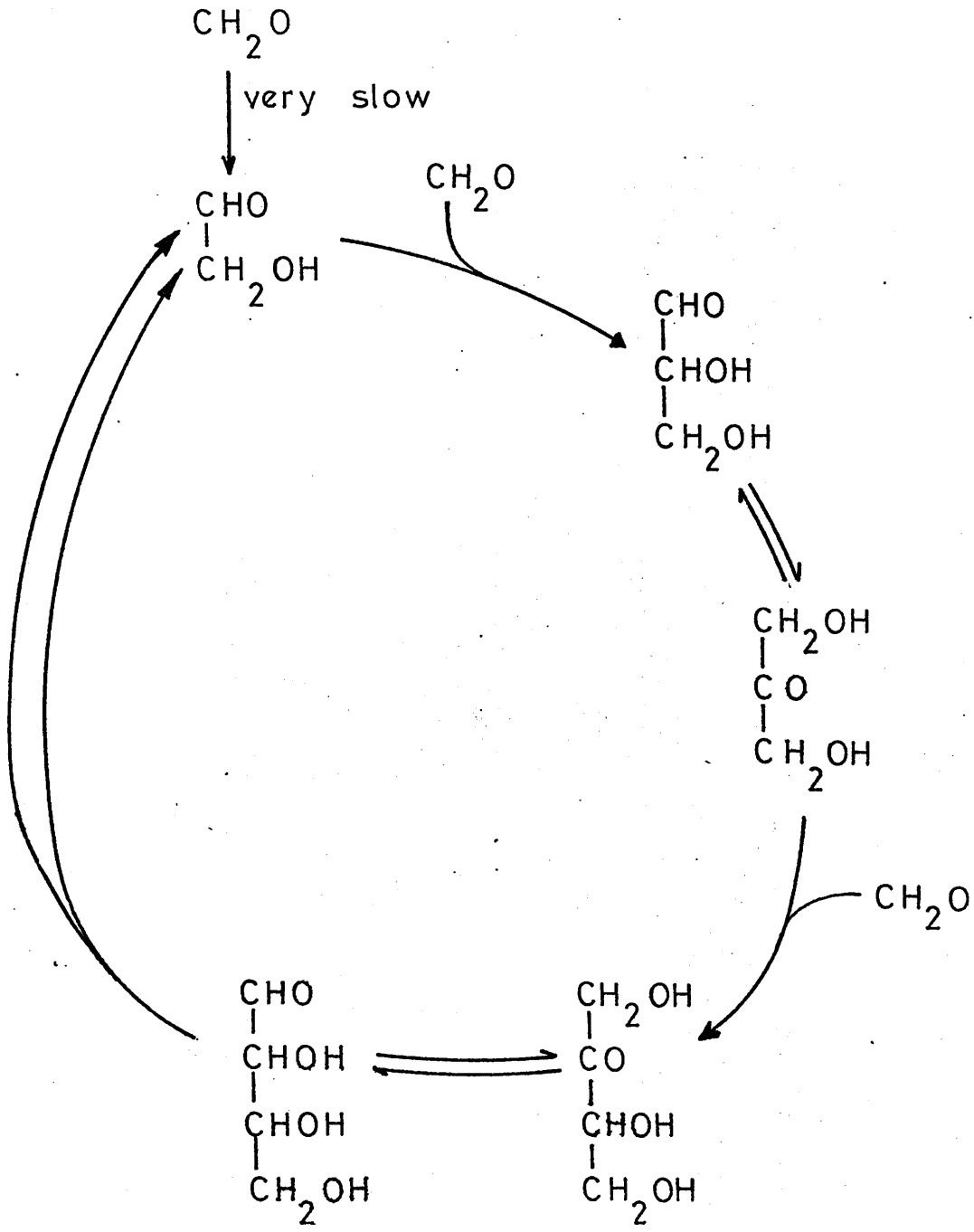
Autocatalytic mechanisms for the aldol condensations.

The first mechanism which gave a possible explanation for the autocatalysis of the second stage of the formaldehyde condensation was composed by Katzschmann in 1944.[69] This is drawn in two ways on the facing page. The top one is the way that Katzschmann published his mechanism: the other represents the same mechanism drawn in a cyclic form to illustrate the reason for the autocatalysis more clearly.

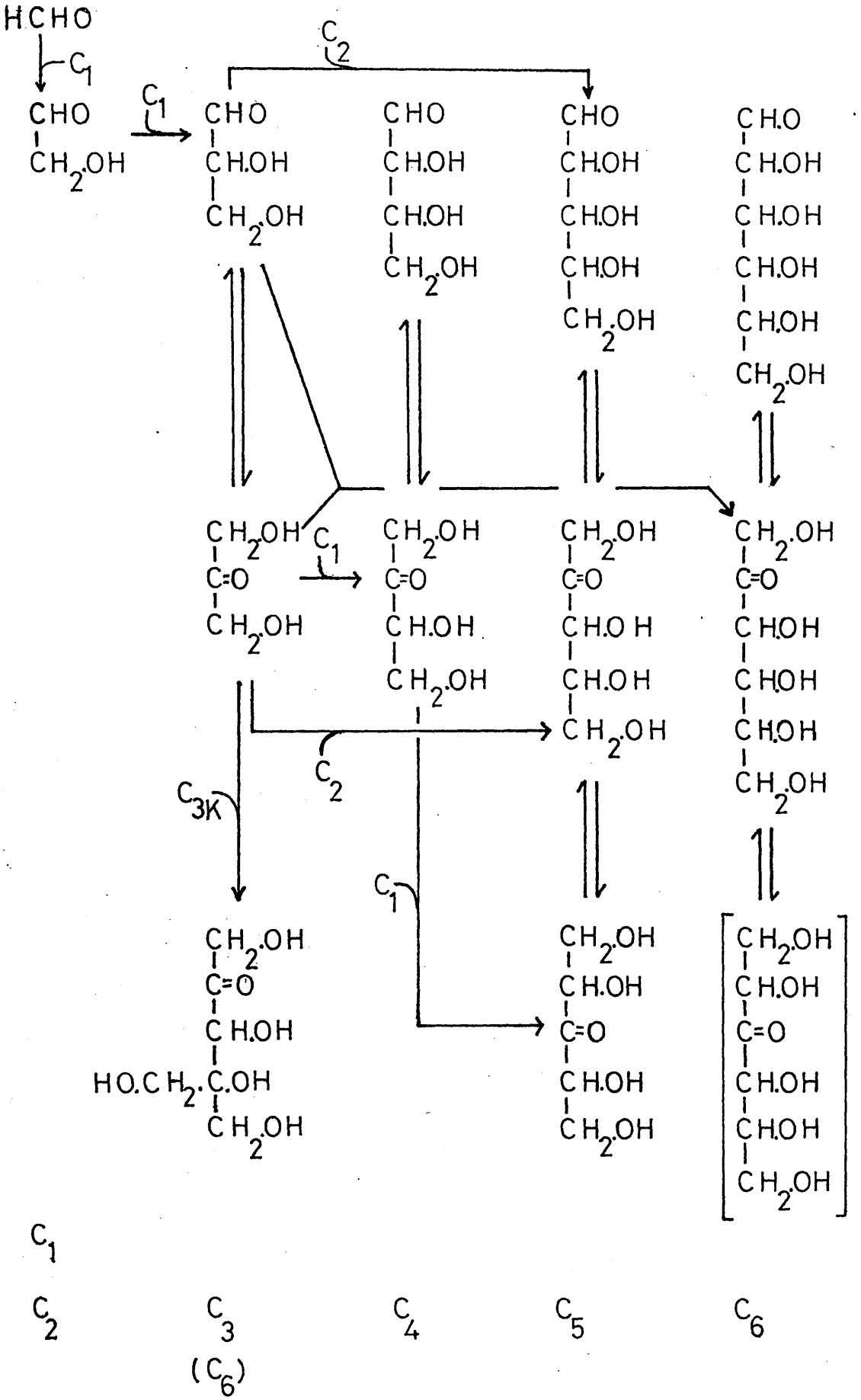
All the reactions in the cycle are aldol condensations or isomerisations except for one, where the cleavage occurs. Katzschmann used bis-hydroxymethyl acetol as his model for the branched sugar in the cycle. (This was prepared from acetol and formaldehyde: both of the formaldehyde molecules added to the same side of the carbonyl group.) It is not easy to see how the last stage of this cycle could have resulted. The products, aldotetrose and glycolaldehyde, cannot undergo aldol reaction to give anything that resembles the bis-hydroxymethyl compound. Nor can their isomers. It seems probable that Katzschmann regarded the branched sugar as a means of activating a single formaldehyde molecule which then reacted with free formaldehyde, rather than the other situation where formaldehyde first added to the branched sugar before it underwent retro-aldolisation.

Activation of formaldehyde (giving an accelerated primary reaction) was referred to by Balezin.[54] Balezin considered that calcium saccharates were formed during the reaction: these united with formaldehyde to give unstable compounds which decomposed to give active formaldehyde. The way in which active formaldehyde is formed by these mechanisms is difficult to find.

THE BRESLOW MECHANISM



THE PFEIL AND RUCKERT MECHANISM



ii The Breslow mechanism.

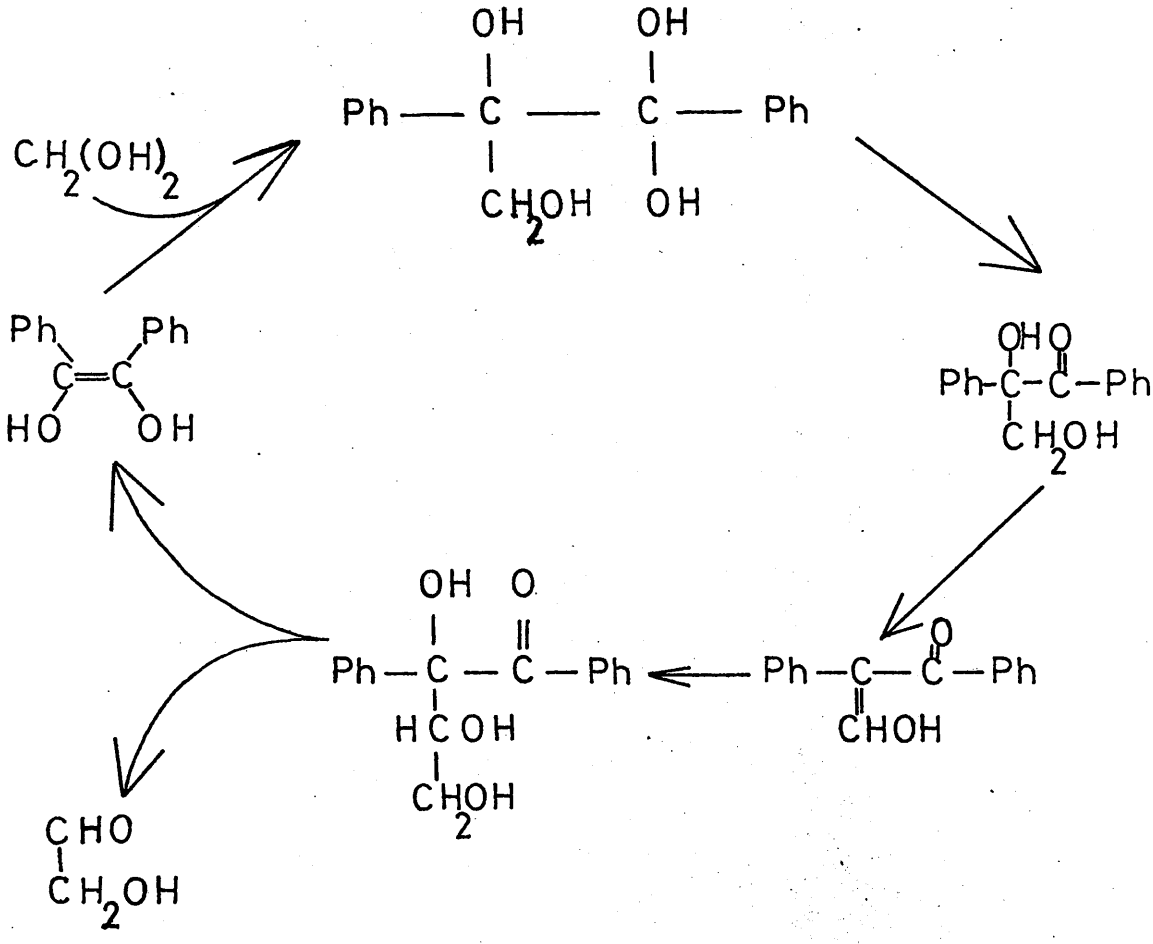
A much more elegant way of explaining the autocatalysis was shown by Breslow in 1959.[93] Here aldotetrose undergoes retro-aldolisation to give two moles of glycolaldehyde (opposite). Breslow suggests that the aldol reactions occur at the usual rate but that the primary reaction is slow because it is an unusual reaction. The mechanism can also show why benzoyl carbinol gives both an immediate and a fast reaction.

iii The Pfeil and Ruckert mechanism.

The most complicated mechanism yet published is Pfeil and Ruckert's (facing). In this many of the pathways may be combined into cycles, by combining aldol, retroaldol and isomerisation reactions.

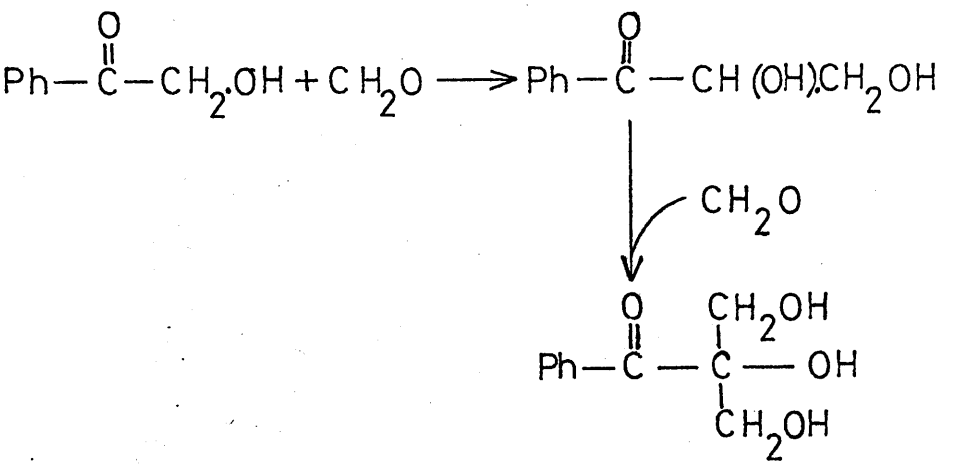
An indication of the complexity of the aldol and other pathways open in the formose reaction is given in Chapter 6.

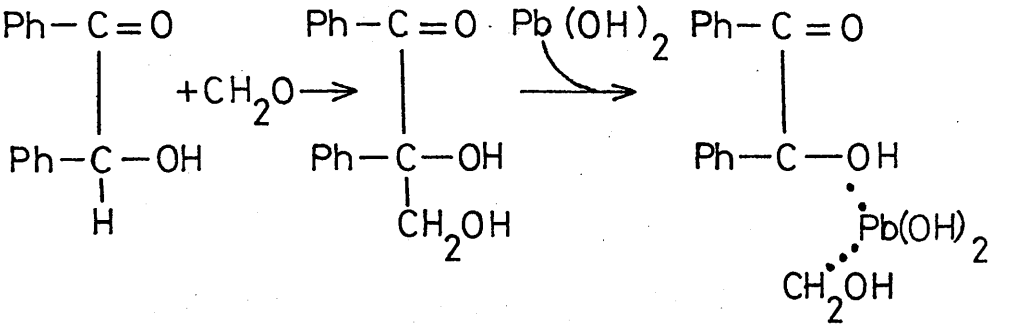
KUZIN'S MECHANISM FOR BENZOIN PARTICIPATION



BENZOYL CARBINOL AND BENZOIN

(Langenbeck)





Mechanisms Involving Non-sugar Accelerators.

Attempts were made to explain the acceleration that benzoin causes by Kuzin[50] and Langenbeck.[91]

Kuzin's mechanism is depicted on one of the facing pages. It consists of a series of condensations and isomerisations which are, for the most part, understandable. The addition of the (second) molecule of formaldehyde to the enone derived from benzoin and formaldehyde is however difficult to justify. Bearing in mind Katzschmann and Balezin's mechanisms, we may be justified in thinking that Kuzin's mechanism also involves the provision of active formaldehyde (this time on an accelerator).

Langenbeck has a similar mechanism to Katzschmann, but involving benzoin and benzoyl carbinol (opposite). Again active formaldehyde is formed. (Langenbeck regards the formose reaction as a true autocatalysis i.e. accelerated primary reaction.[63])

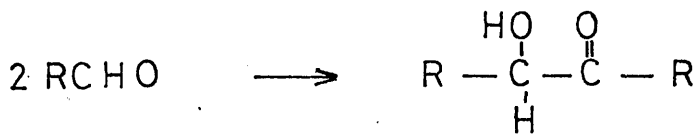
Many of the other non-sugar accelerators were viewed in the same light e.g. vitamin C and ethoxyacetaldehyde. We can see that the involvement of the latter would allow Kuzin to support the active formaldehyde role rather than the aldol role.

The involvement of benzoin, ascorbic acid and some other accelerators will be discussed in the next chapter.

THE UNIQUENESS OF THE FORMOSE REACTION

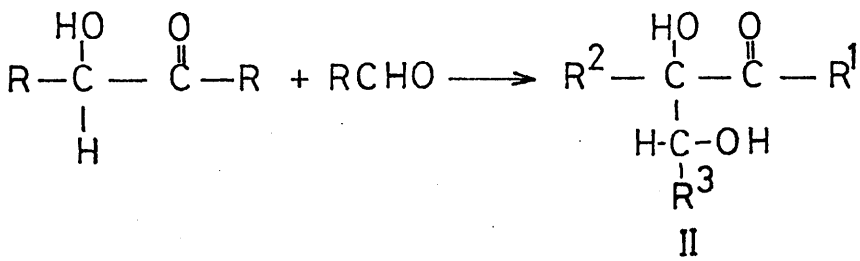
Why should the formose reaction be unique? Let us consider what the requirements for this reaction are.

We shall start with aldehyde R.CHO, where R is a group that cannot undergo aldol condensation e.g. phenyl. Let two moles of this aldehyde combine to give acyloin I.



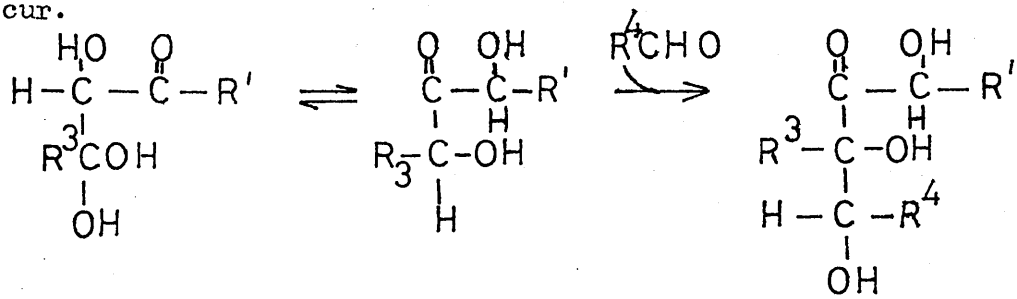
I

I possesses an active hydrogen atom, which was one of the orginial aldehydic substituents. This compound may undergo aldol condensation with another mole of aldehyde to give II.



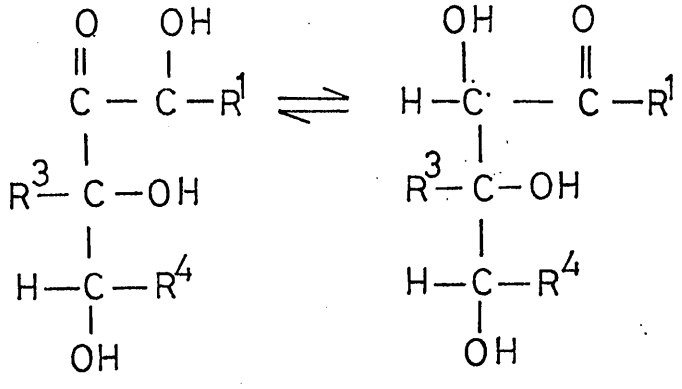
II

In II there are no further active hydrogen atoms on carbon. Let us label the R groups R^1, R^2, and R^3. If further reaction is to occur, then the hydrogen atom on carbon 3 must be activated. This can only be done by the presence of an α-carbonyl group, hindered at the moment because of R^2. Let us replace R^2 by H: then the following isomerisation and condensation may occur.



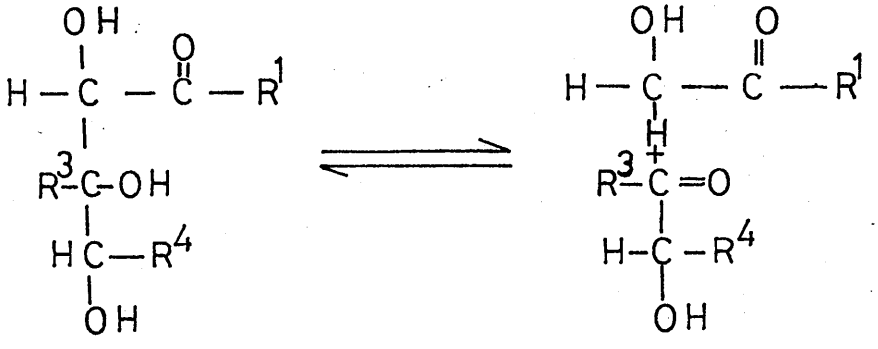
II′ III IV

Alternatively, if the carbonyl group has not moved from carbon 1 to carbon 2, aldol condensation may occur between carbon 2 and a further mole of aldehyde, R.CHO. This results in carbon 2 becoming tertiary and no further reaction is possible. (We can see the similarity to Katzschmann's intermediate.)



IV V

IV may isomerise to V, where the carbonyl has returned to its original position. V can retro-aldol to II and II'.



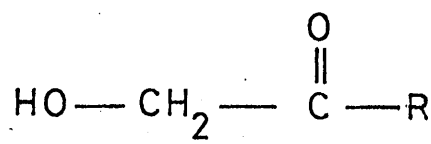
We have now the miniumum requirements for a cyclic mechanism.

If the starting aldehyde is benzaldehyde, say, no cyclic mechanism is possible. If the starting aldehyde is formaldehyde, then we can have a cyclic mechanism. If the starting aldehyde contains a little formaldehyde, then a non-autocatalytic cycle can start. (Benzoyl carbinol, an example of the mixed aldehyde, is a known accelerator of the formose reaction.) This may

even be used as a route for the preparation of acyloins without the necessity of cyanide: here the carbinol can be prepared from formaldehyde and whatever aldehyde is intended to undergo acyloin formation.

If benzoyl carbinol is added to formaldehyde then the subsequent reaction will be autocatalytic, because of glycolaldehyde formation (R^3 and R^4 = H). Glycolaldehyde will establish a Breslow cycle.

The uniqueness of the formose reaction lies in its ability to form an acyloin with an active methylene.[93]



FUTURE DEVELOPMENTS OF THE FORMOSE REACTION.

No complete analysis of <u>all</u> of the products of the formose reaction has yet been attempted. Even the analysis of the sugar fraction alone might show that a large number of branched sugars are formed. The application of gas-chromatography and mass spectrometry to this problem may prove invaluable.

The precise mechanism for the primary reaction has yet to be established: the various physical methods of structure analysis (N.M.R. and U.V. spectroscopy, etc.) that are in routine use in other branches of organic chemistry, have yet to be applied to the formose reaction.

An increasingly important use of the formose reaction is in the supply of food in a small environment. The nutritional value of formose has been included in Appendix VI.

Patents covering aspects of the formose are reviewed in Appendix V.

CHAPTER 2

THE ALUMINA-CATALYSED FORMOSE REACTION

Introduction.

In Chapter 1 we saw that there were differing reports as to whether some compounds could catalyse the formose reaction. Taking a specific example, Schmalfuss[39] found that aluminium hydroxide was not a catalyst. Malinowski came to the same conclusion about alumina.[94] Franzen was unable to isolate a formaldehyde salt of aluminium.[33,34] Gabel and Ponnamperuma, on the other hand, isolated triose, tetrose, pentose and hexose as a result of alumina catalysis.[119*]

A number of other points were raised in that chapter, such as the effect of cyanide ion on the primary reaction, and the role of non-sugar accelerators.

In order to answer some of these questions, the alumina-catalysed formose reaction was examined, and as it proved to be satisfactory, was used as a system for investigating various aspects of the formaldehyde condensation.

Discussion.

Formaldehyde was refluxed in the presence of neutral aluminium oxide under conditions broadly similar to those employed by Gabel and Ponnamperuma[119](Ia). After three hours the odour of caramel was detected, and the solution and the alumina

* We shall not deal with mixed, alumina-containing catalysts.

TABLE Ia

PRODUCTS FROM NEUTRAL ALUMINA (Aniline Oxalate Spray)

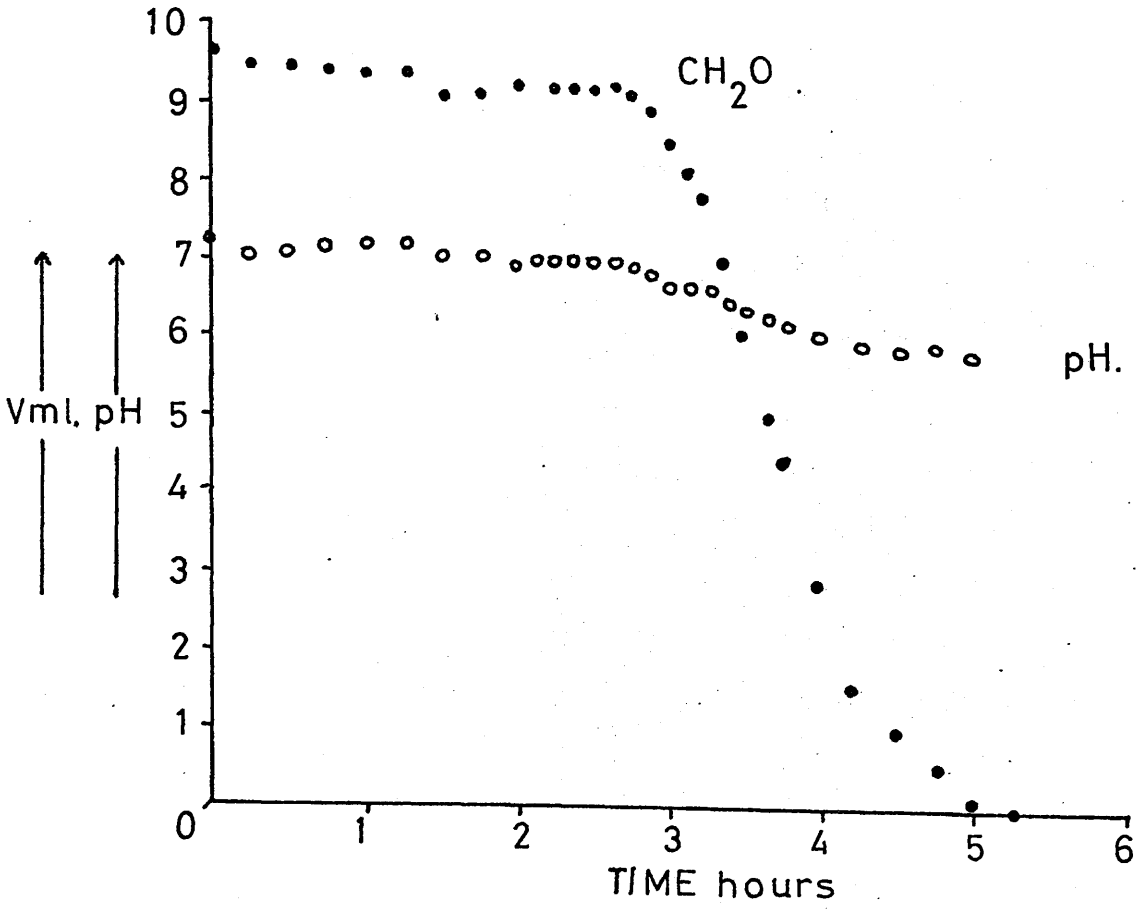
FORMOSE

$R_{glucose}$	COLOUR visible light	COLOUR ultraviolet light	IDENTITY
1.0_0	yellow	salmon/brown	$C_{6(A?)}$
1.1_6	pink	pink	C_{5A}
1.45	colourless	violet	unknown (caramelisation product?)
1.6_5	yellow	yellow	C_4
1.7_7	orange	orange	C_{3K}
2.0_0	yellow (wk.)	blue	$C_{3A?}$
2.2_5	yellow (wk.)	yellow	$C_{2A?}$

wk. - weak

Fig. II

GRAPHS OF FORMALDEHYDE CONCENTRATION AND pH AGAINST TIME



were beginning to turn yellow. Refluxing was continued for a further two hours, after which no formaldehyde remained. Products identical in behaviour to sugars, ranging from C_2 to C_6 (as regards their paper chromatographic mobilities and colour reactions with several sprays), were formed. The colours and $R_{glucose}$ values of the spots observed after the use of the aniline oxalate spray are recorded on the opposite page. No compounds of $R_{glucose}$ values less than 1.00 were found, although there was some minor streaking.

Neutral alumina exerts a pH of approximately 7.4 in aqueous slurry. The possibility that hydroxide ion alone catalysed the reaction was dismissed by refluxing sodium hydroxide solution at that pH with formaldehyde (Ib). Extraction of the aluminium oxide under similar conditions with water failed to show the presence of formaldehyde or sugar (Ic).

Having shown that alumina can catalyse the formose reaction we are now able to consider the variation of the formaldehyde concentration during the reaction's course. The conditions used in the first experiment were repeated on a larger scale, and both the formaldehyde concentration and the pH were measured (Fig.II, Experiment II). The shape of the aldehyde curve is what we would have expected, showing a lag period before the rapid decrease in concentration. The pH remained approximately constant during the reaction, but a slight fall was observed, the rate of decrease being greatest after the end of the induction period. This may be due to the cross-Cannizzaro reaction between formaldehyde and the sugars. The rate of fall of pH was greater after 4 hours than at the start of the reaction, possibly because of caramelisation of the sugars.

Because of the large amount of alumina present in Experiment II (100g.), stirring had to be used to agitate the alumina, rather than allow a "cake" to form in which the availability of formaldehyde would have been diffusion controlled.

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.III

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA.
under nitrogen, with stirring.

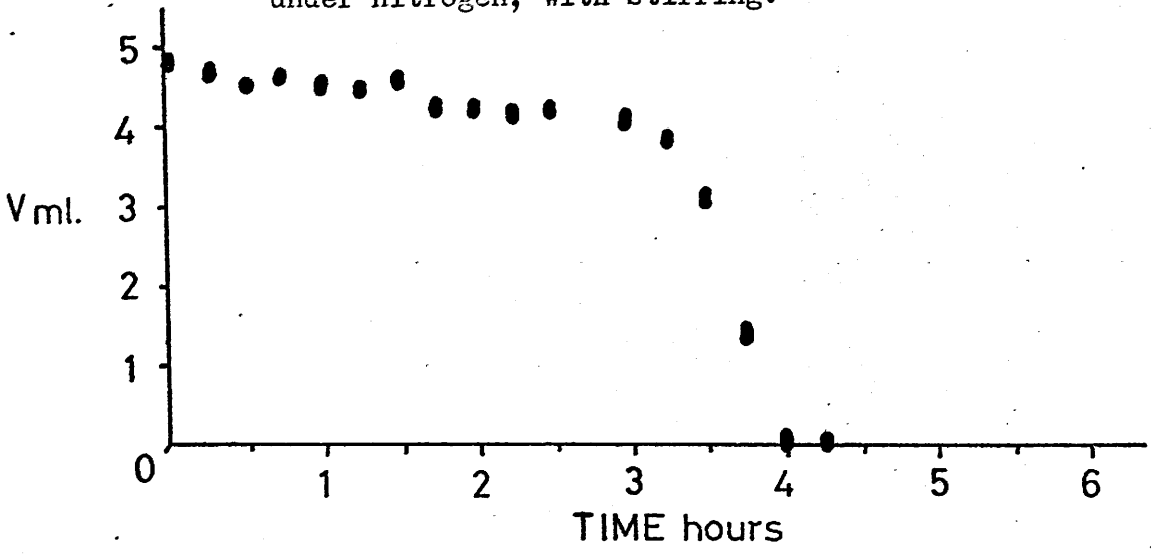
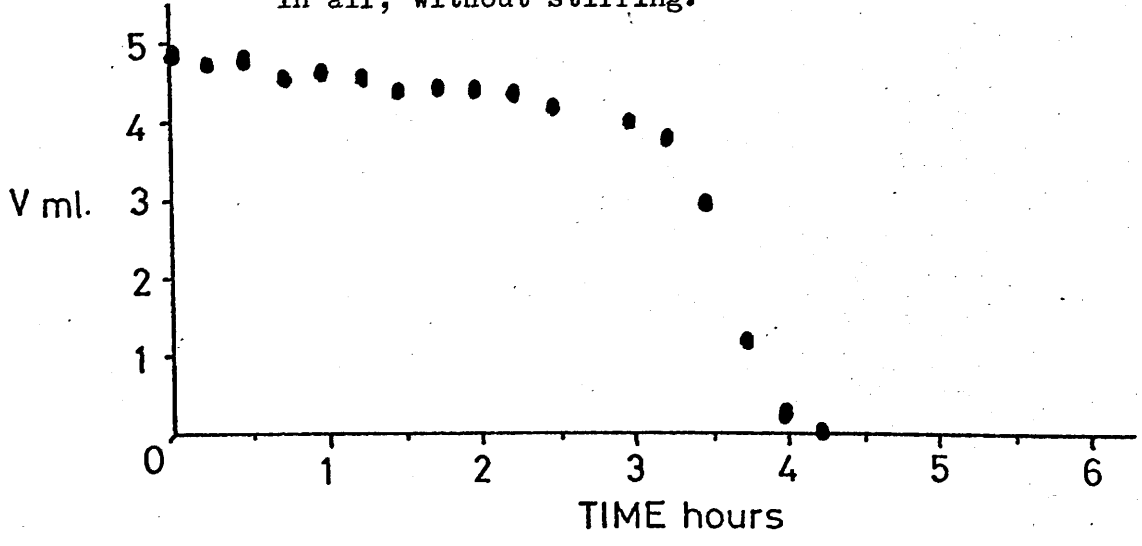


Fig.IV

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA.
in air, without stirring.



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.Va, b

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 1mg. accelerator.

a. D-glyceraldehyde (•)

b. 1,3-dihydroxyacetone (o)

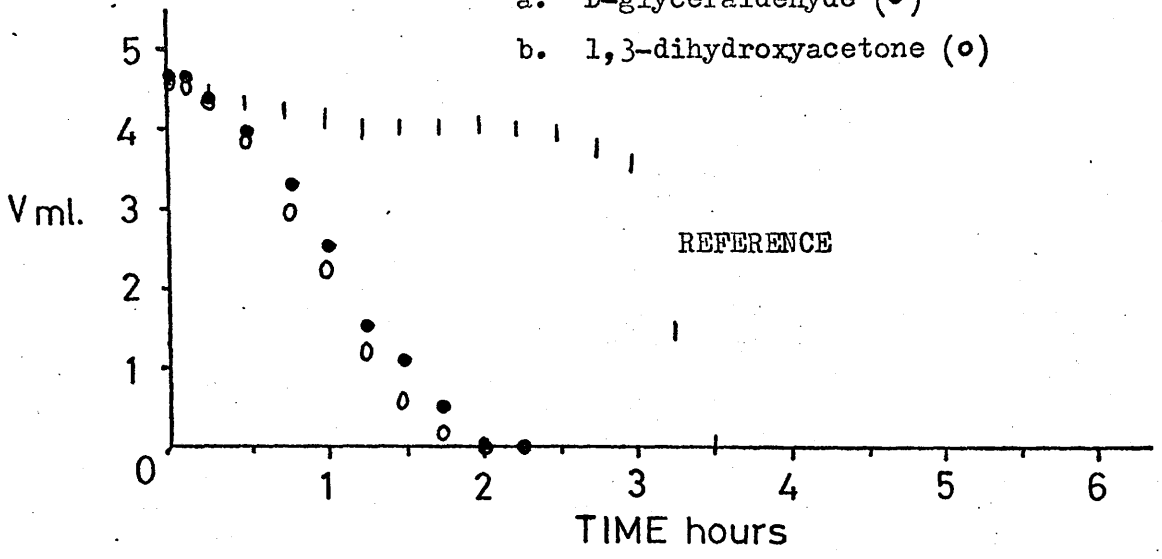
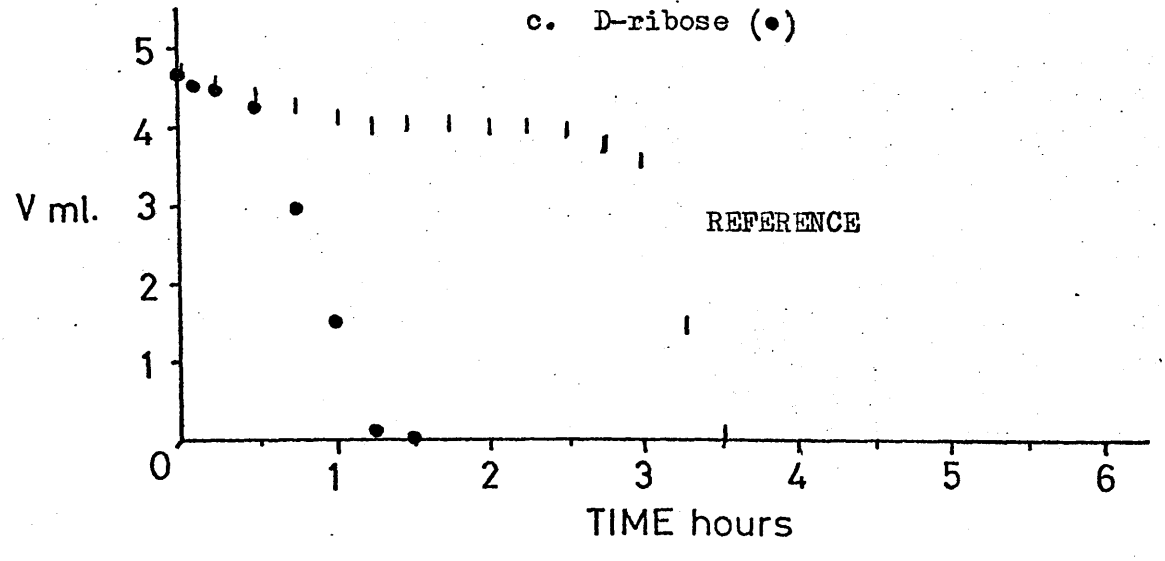


Fig.Vc

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 1mg. accelerator.

c. D-ribose (•)



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.V d,e

30g. n-Al_2O_3 + 150ml 0.13M aq. PFA. + 1mg. accelerator.

d. D-glucose (•)

e. D-fructose (o)

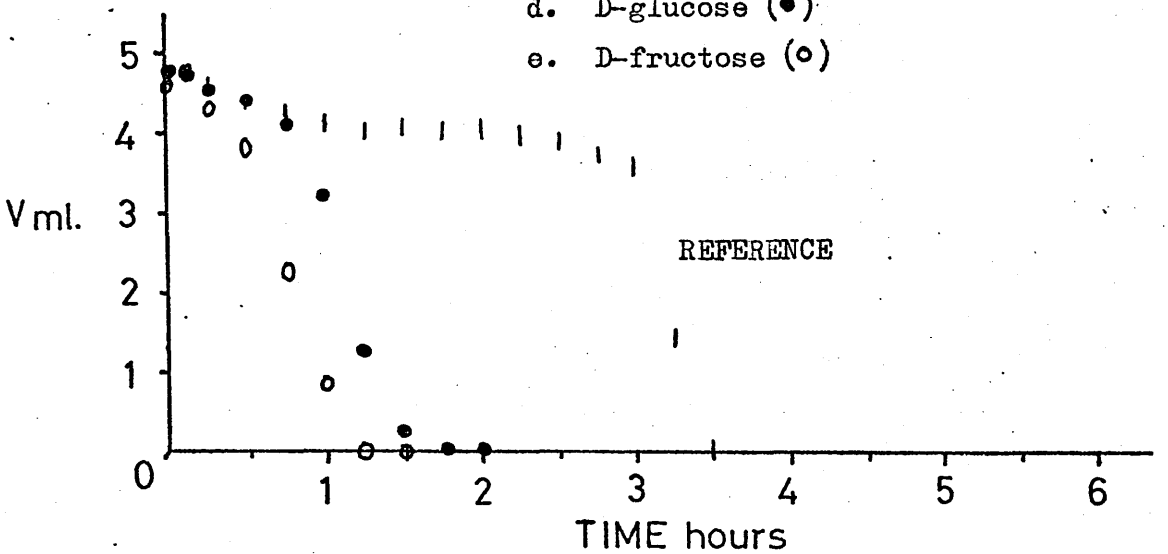


Fig. V f,g

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 1mg. accelerator.

f. sucrose (•)

g. soluble starch (o)

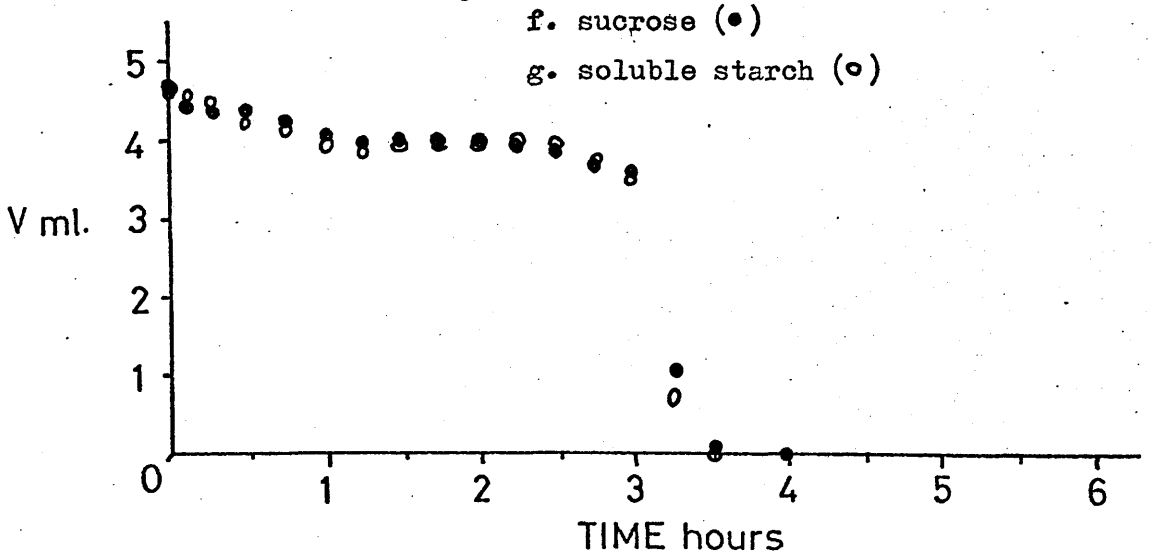
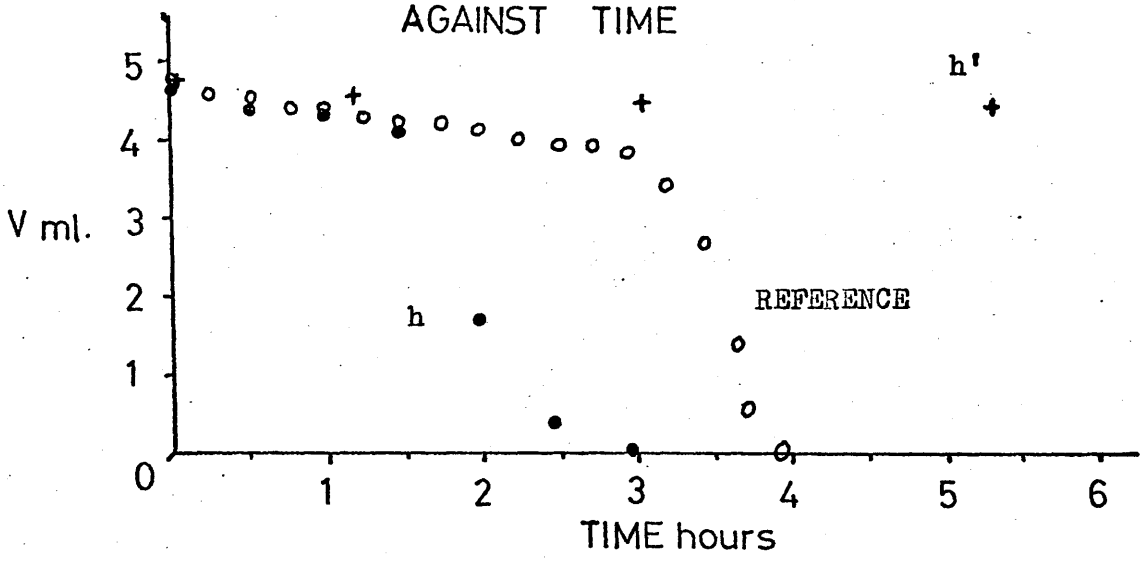


Fig. V h,h'

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME

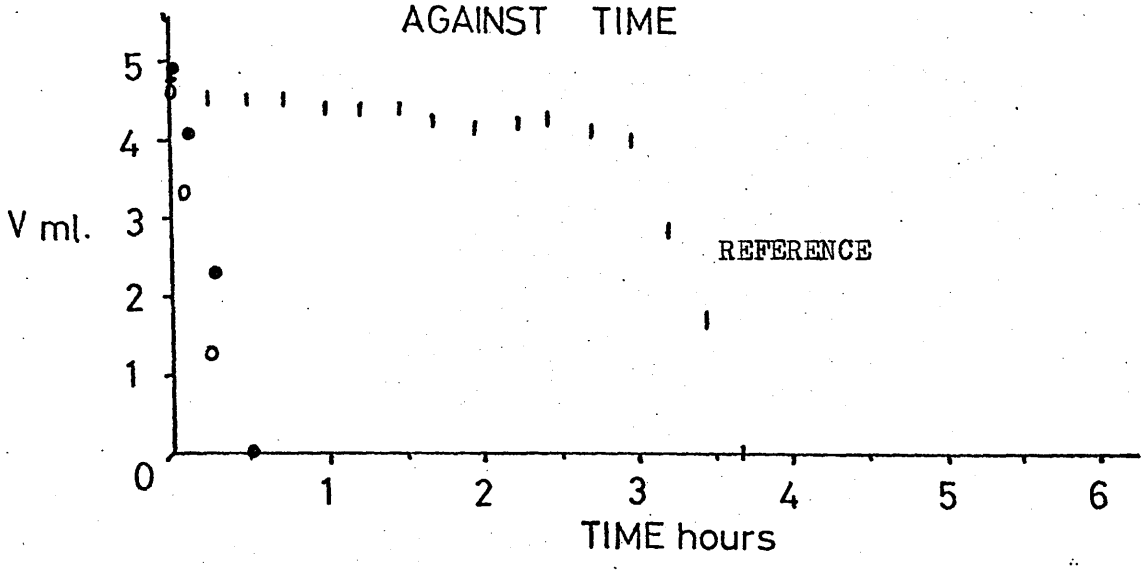


h. 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + ca. 4mg. formose (•)
(in 1 ml. solution)

h'. 150ml. 0.13M aq. PFA. at pH 7.4 (NaOH/HCl) (+)
+ ca. 4mg. formose (in 1 ml. solution)

Fig.VIa,b

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



a. 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 3.6g. D-glucose (•)

b. 30g. n-Al_2O_3 + 150ml. 0.13m aq. PFA. + 18g. D-glucose (o)

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.VIc

30g. n-Al_2O_3 + 150ml. 0.13M aq.PFA. Refluxed for 24 hrs. prior to the addition of 600mg. PFA.

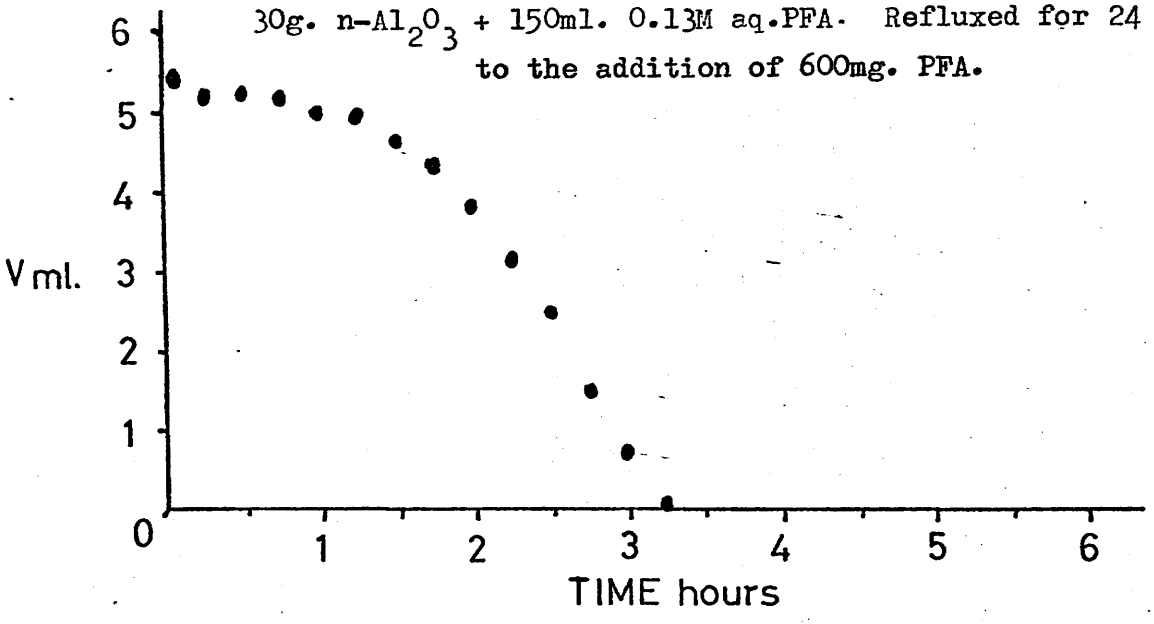
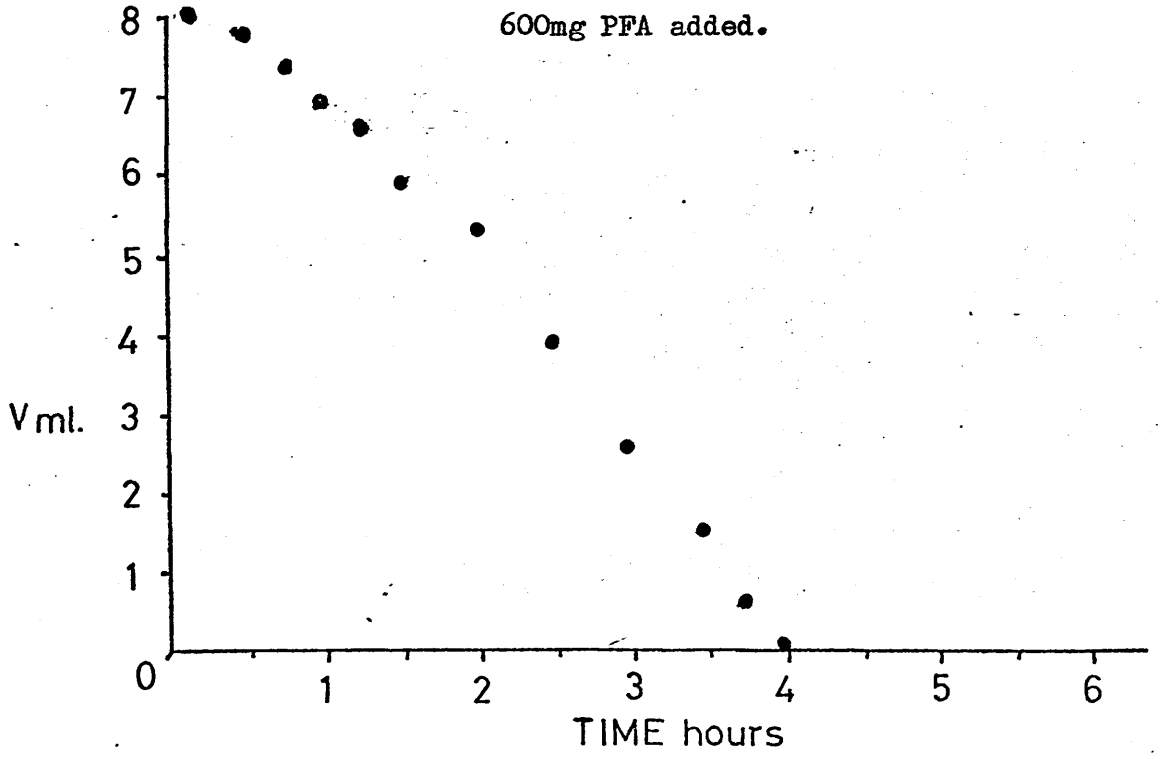


Fig. VId

The product from VIc was heated for 15 hours and a further 600mg PFA added.



The reaction was repeated on a reduced scale, with 30g. alumina, and without stirring. (Here thermal energy was sufficient to agitate the alumina.) The formaldehyde uptake was at almost the same rate (Fig.III). Removal of the nitrogen atmosphere also had little effect (Fig.IV), showing that atmospheric oxidation of the formaldehyde was negligible.

The conditions of Experiment IV will be used in many of the following experiments. They will be considered as the "standard" conditions. A slight variation in the shape of the curve of formaldehyde concentration for this system was found: consequently where a comparison has to be made of one curve with the standard, a "reference curve" has been provided, obtained from the same batch of alumina, etc.. This curve has not been drawn where the new curve coincides with the reference, for the sake of clarity.

Do sugars accelerate the alumina-catalysed formose reaction? A selection of aldose and ketose sugars were added in small amounts to the standard system, and most of them were found to cause an acceleration (Figs.Va-g). Those that did not, sucrose and soluble starch, did not have ene-1,2-diols. Formose accelerated the reaction (Fig.Vh). Control experiments with sodium hydroxide at pH 7.4 were performed, and in no case was any formose formed (Va'-h'). The formaldehyde concentration curve obtained from formose was typical (Fig.Vh'). The presence of alumina is therefore necessary for sugars to be formed (in a short time

Does a large amount of sugar inhibit the formose reaction as Pfeil[82] suggested? An equimolar amount and a five-fold molar excess of D-glucose were found not to have an inhibitory effect (Fig.VIa,b). Indeed, rapid uptake was observed in both cases. Similarly, formose and alumina mixtures continued consume portions of formaldehyde without sign of inhibition (Fig.VIc,d). (The formaldehyde concentrations are successively higher because of the decrease in volume from aliquot removal.)

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.VIIa,b

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + accelerator

a. 0.01mg D-fructose* (o)

b. 0.001mg. D-fructose* (•)

(The curve with 1 mg. D-fructose* is repeated: V e.)

* Added to the solution.

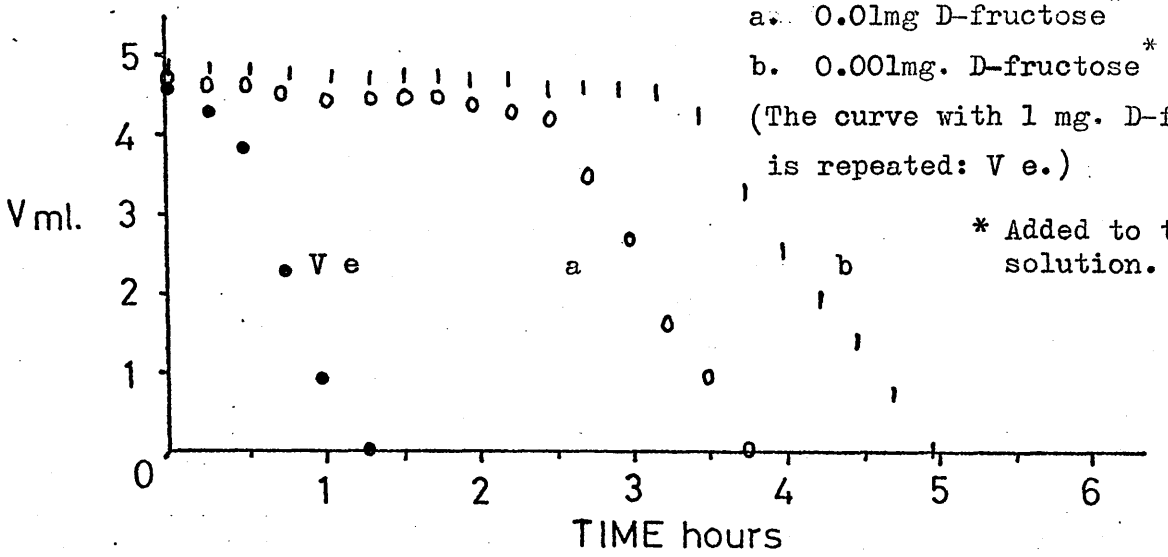


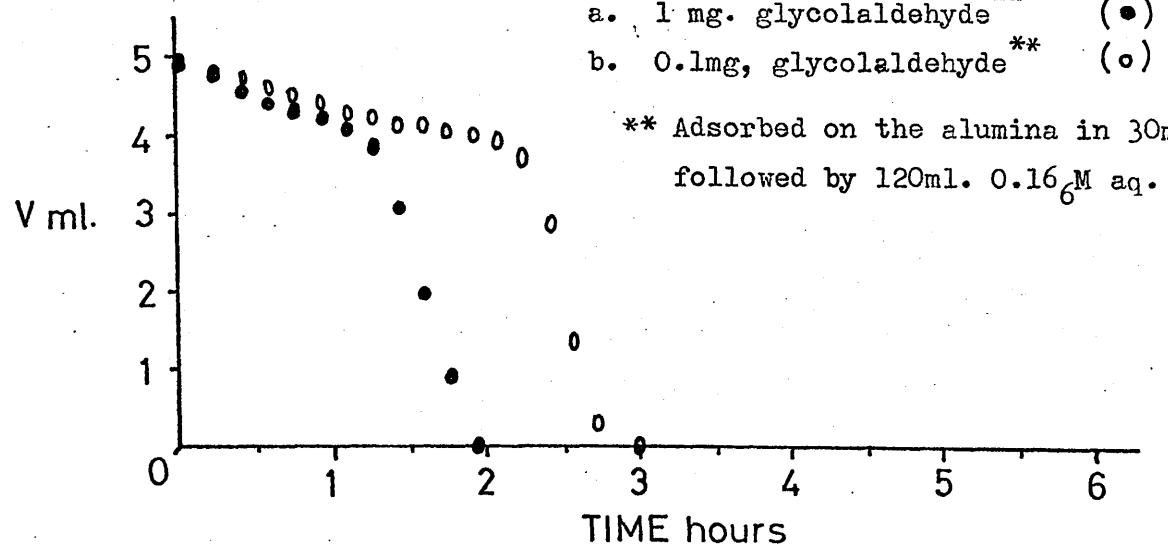
Fig.VIIIa,b

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + accelerator

a. 1 mg. glycolaldehyde** (•)

b. 0.1mg, glycolaldehyde** (o)

** Adsorbed on the alumina in 30ml. H_2O followed by 120ml. 0.16_6M aq. PFA.

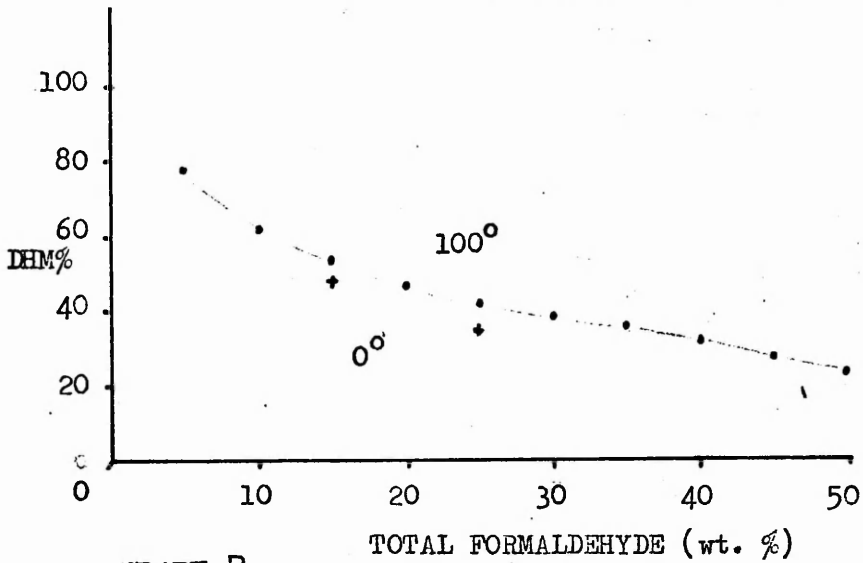


Of the sugars examined for their accelerating ability, fructose was found to be the best, a conclusion recently reached independently by Nakai.[128] What effect will there be on decreasing the amount of fructose? One-hundredth and one-thousandth of the quantity of fructose that had been added earlier (Ve) were found to cause lesser reductions in the lag period (Fig.VIIa,b). A similar effect was observed with glycolaldehyde (Fig.IXa-c). As it was possible that when 0.001mg. accelerator was added to the solution little of it was actually adsorbed on the alumina, the experiment was repeated by first allowing the glycolaldehyde to adsorb on the alumina prior to the addition of formaldehyde. The adsorption was carried out at room temperature, consequently, after the formaldehyde had been added, some time was taken for the solution to attain reflux temperature. This makes a direct comparison difficult, but one is possible if the differences in the times of completion of the reaction, for comparable amounts of accelerator are used (Figs.VIIIa-d,IXa-c). The differences in times of completion of the reaction between amounts of accelerator that differ by ten-fold are similar in both cases, being approximately three-quarters of an hour. There would also appear to be a similar logarithmic relationship between ten-fold amounts of fructose. (If the data for fructose is interpolated, the time of completion of the reaction in which 0.1mg. was involved, would be expected to be $2\frac{1}{2}$ hours.)

If Figs. VIIIc and d are compared this relationship does not hold. If we assume that the reason for this is that 0.001mg. has no accelerating effect i.e. the latter Figure corresponds to the reference curve, the implication is that adsorption of 0.01mg. of glycolaldehyde has a lesser effect than addition of the same amount to the solution. The reason for this is not immediately clear, but it may be due to loss of accelerator by cross-Cannizzaro reaction at lower temperatures, or some other means of destruction of the accelerator.

GRAPH A

GRAPH OF PERCENTAGE OF DISSOLVED FORMALDEHYDE AS DIHYDROXYMETHYLENE (DHM)

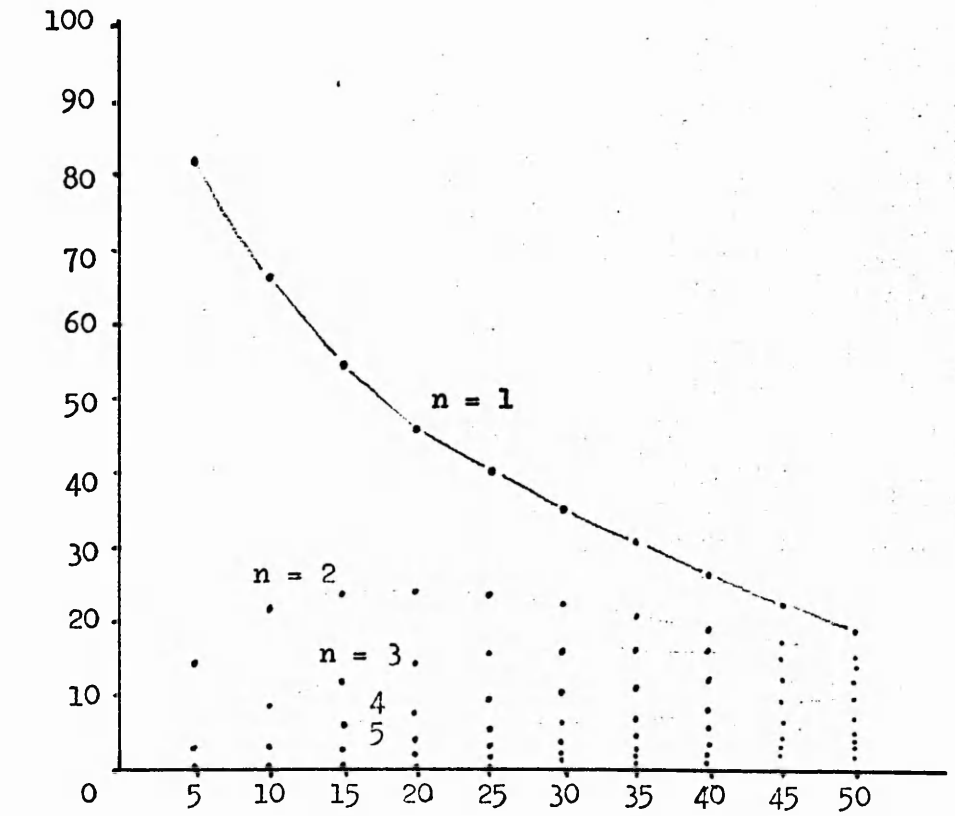


TOTAL FORMALDEHYDE (wt. %)

(After J.F Walker, loc.cit.,67.)

GRAPH B

DISTRIBUTION OF FORMALDEHYDE POLYMERS IN AQUEOUS SOLUTION AT 35°



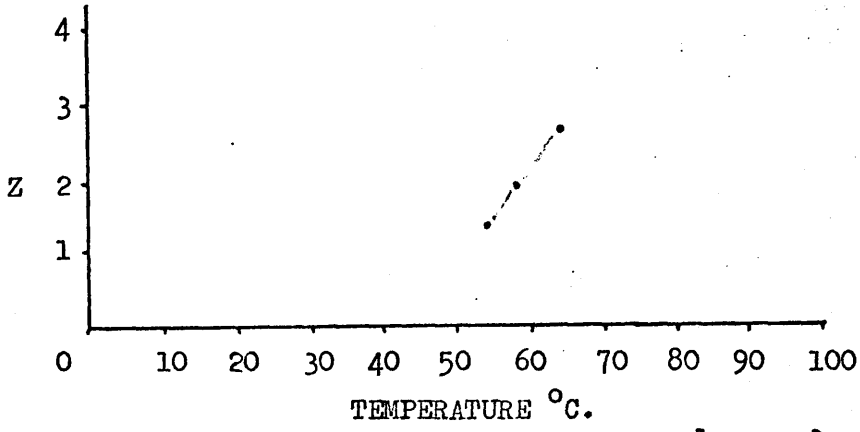
% CH_2O AS POLYMER WITH n CARBON ATOMS

(After J.F. Walker, loc.cit.,64.)

GRAPH C

FORMALDEHYDE MONOMER CONTENT FOR

0.87M AQUEOUS FORMALDEHYDE

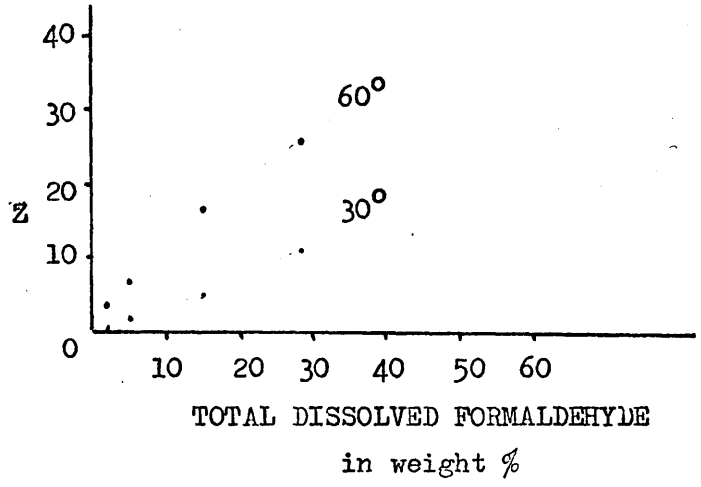


(After J.F. Walker, loc. cit., 54.)

Z is the monomer content in moles litre^{-1} x 10^{-3}

GRAPH D

APPROXIMATE FORMALDEHYDE MONOMER CONTENT OF AQUEOUS SOLUTIONS OF FORMALDEHYDE



(After J.F. Walker, loc. cit.,61.)

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.VIII c,d

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + accelerator

c. 0.01mg. glycolaldehyde** (●)

d. 0.001mg glycolaldehyde** (o)

** Adsorbed on the alumina in 30ml. H_2O: followed by 120ml. 0.16_6M aq. PFA.

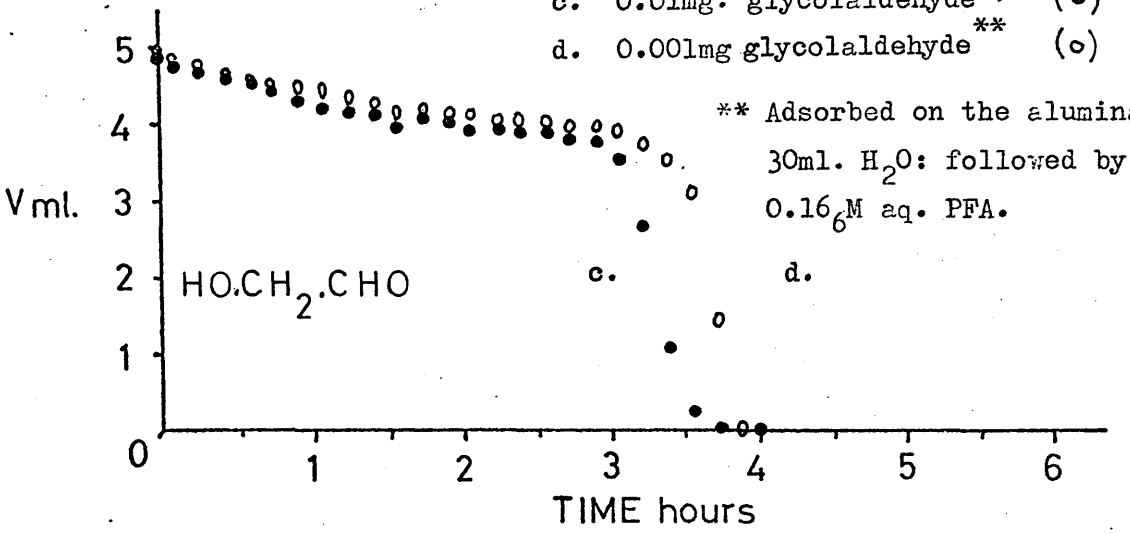


Fig. IX a,b,c

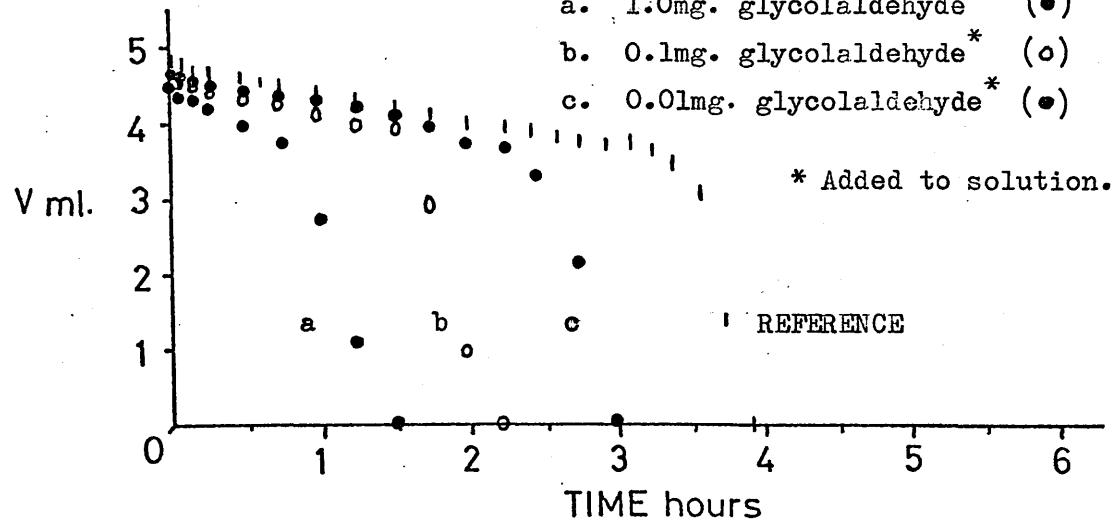
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + accelerator

a. 1.0mg. glycolaldehyde* (●)

b. 0.1mg. glycolaldehyde* (o)

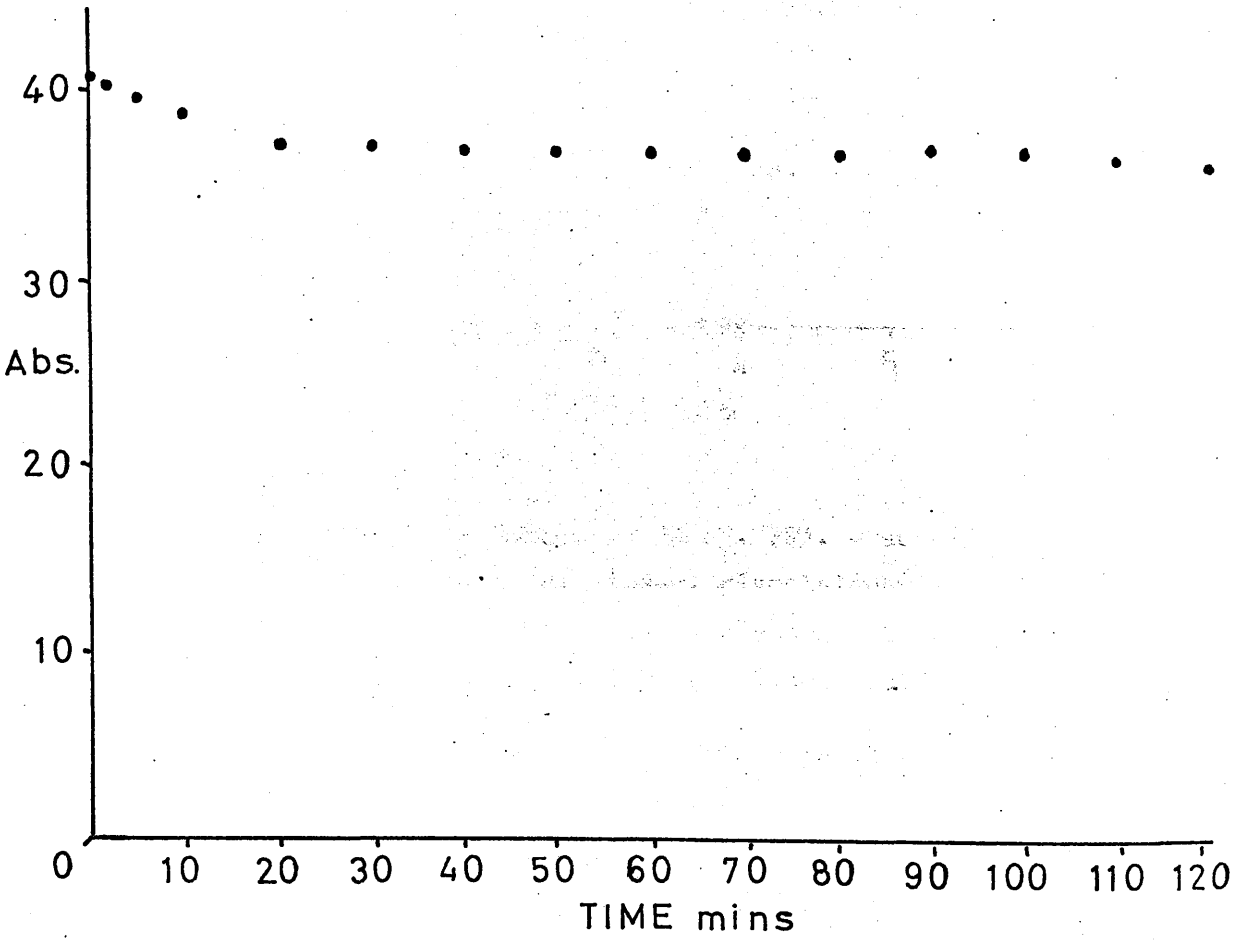
c. 0.01mg. glycolaldehyde* (●)

* Added to solution.



GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. X



(The Cannizzaro reaction only may occur at lower temperatures, the formose reaction commencing only when the temperature is sufficiently high. See Appendix IV.) Another explanation for this might be that the glycolaldehyde was adsorbed with deactivation on the alumina surface.

The Composition of 0.13M Aqueous Formaldehyde Solution.

The approximate composition of the formaldehyde solution with which we are dealing can be found from the accompanying Graphs (A, B). A 0.13M solution contains 0.4% w/v formaldehyde. The solution is composed almost entirely of dihydroxymethylene. The monomer (anhydrous formaldehyde) concentration is low, but rises with temperature (Graphs C, D).

These graphs refer to aqueous solution at an unknown pH, but it would be expected that they would be generally applicable to the systems with which we are dealing. The presence of cations might enable a high concentration of the monomer as a ligand to be attained.

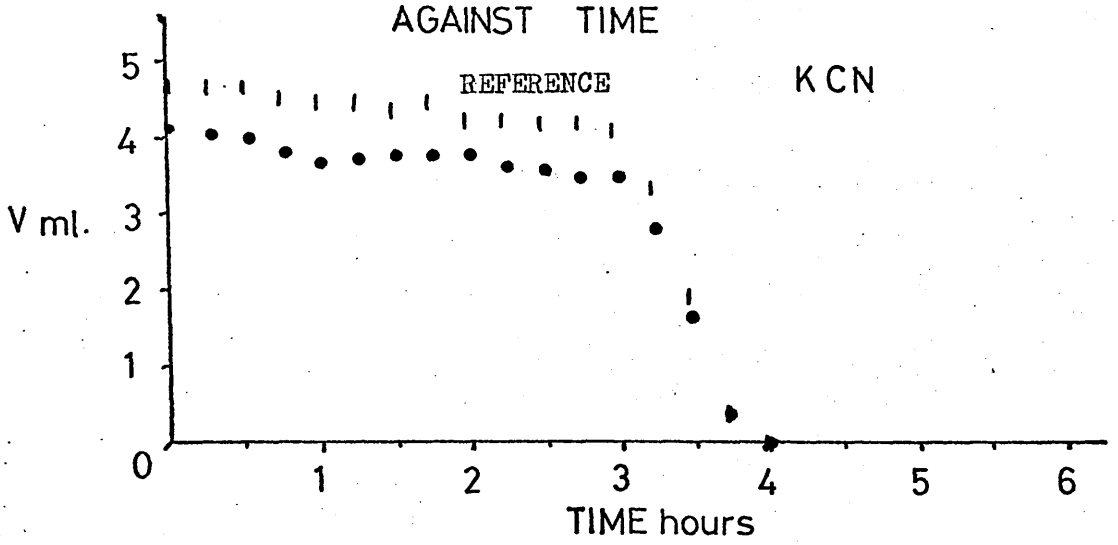
$$H_2C{=}O\cdots\cdots M^{n+}$$

Adsorption of Formaldehyde on Alumina.

Some of the earlier graphs show a small rapid decrease in the formaldehyde concentration. This may be due to adsorption on the alumina. The formaldehyde concentration was measured at frequent intervals during the early stages of the reaction (Fig.X). The decrease was approximately 10% in 30 minutes. (The formaldehyde was estimated by the chromotropic acid method.)

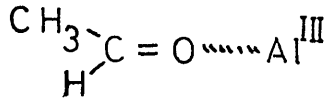
Fig.XI

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



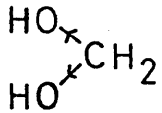
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 130mg potassium cyanide (●)

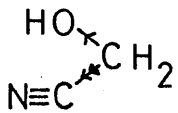
It is known that acetaldehyde can be adsorbed onto alumina.[103]



The Effect of Cyanide on the Alumina-catalysed Formose Reaction.

Formaldehyde and hydrogen cyanide form formaldehyde cyanohydrin almost quantitatively. The ionisation of the methylenic hydrogen atoms in formaldehyde cyanohydrin would be expected to be easier than in formaldehyde hydrate.





The Gabel and Ponnamperuma "hydrate" mechanism involves the ionisation of dihydroxymethylene on the alumina surface (Chapter 1). We might therefore expect that the addition of cyanide would encourage the ionisation, and would reduce the lag period of the reaction.

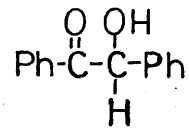
Sufficient potassium cyanide to convert 10% of the formaldehyde to the cyanohydrin, was added to the standard quantities of formaldehyde and alumina. The formaldehyde concentration was estimated as usual (Fig.XI). (The formaldehyde readings are correspondingly low because of the cyanohydrin.) The induction period was not reduced. From this we may conclude that either the hydrate mechanism is not applicable to this system, or that the acceleration conferred by the cyanide is balanced by a deceleration from loss of co-ordination of the second oxygen to the alumina.

The effect of the addition of cyanide has proved to be more marked in the sodium hydroxide-catalysed reaction (Chapter 3).

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig.XIIa

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA + 42.5 mg. benzoin (•)



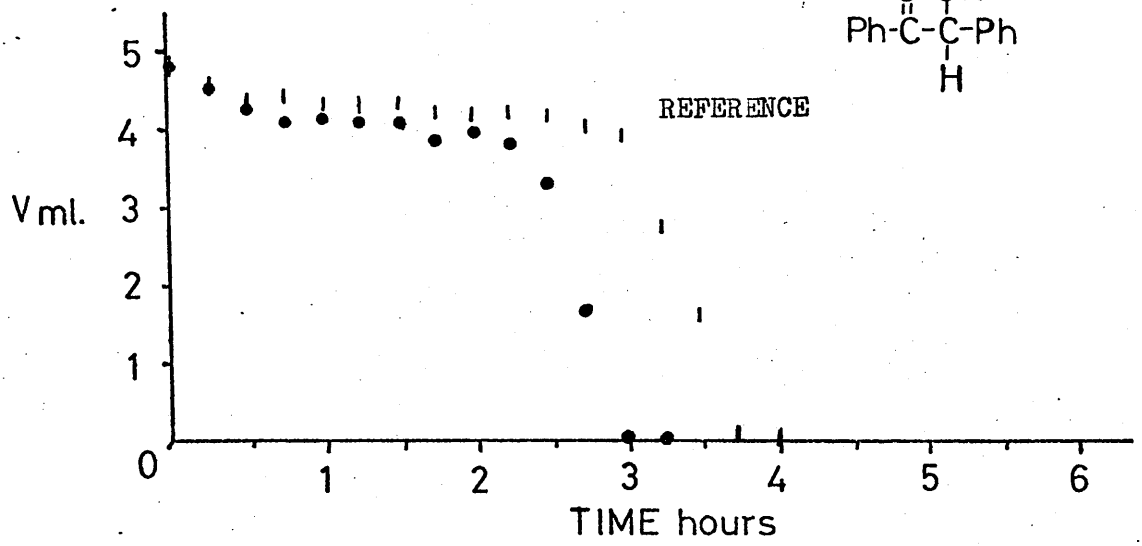
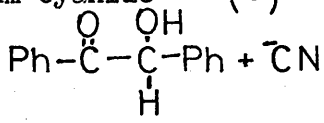
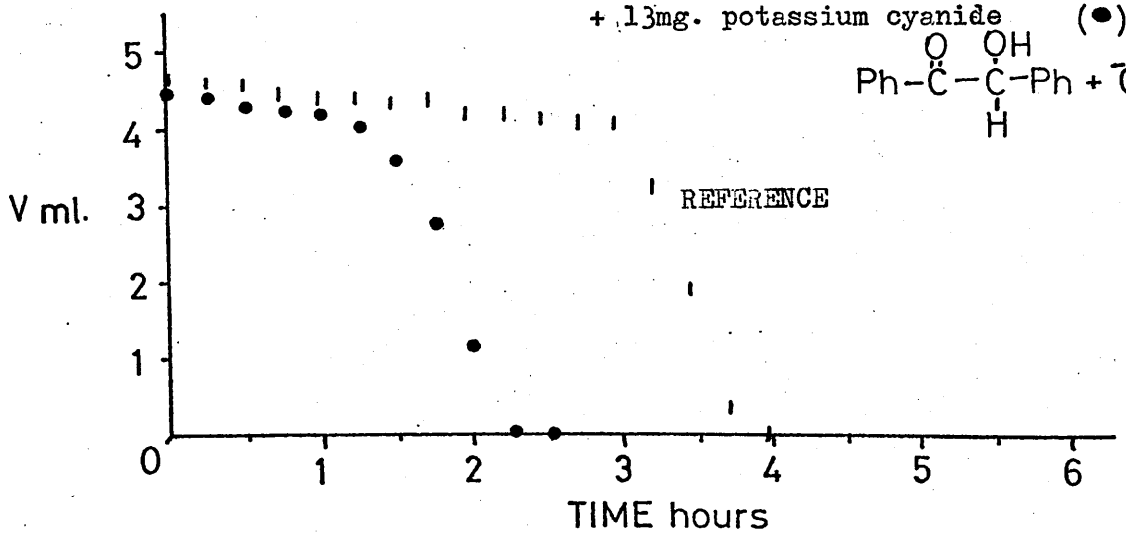


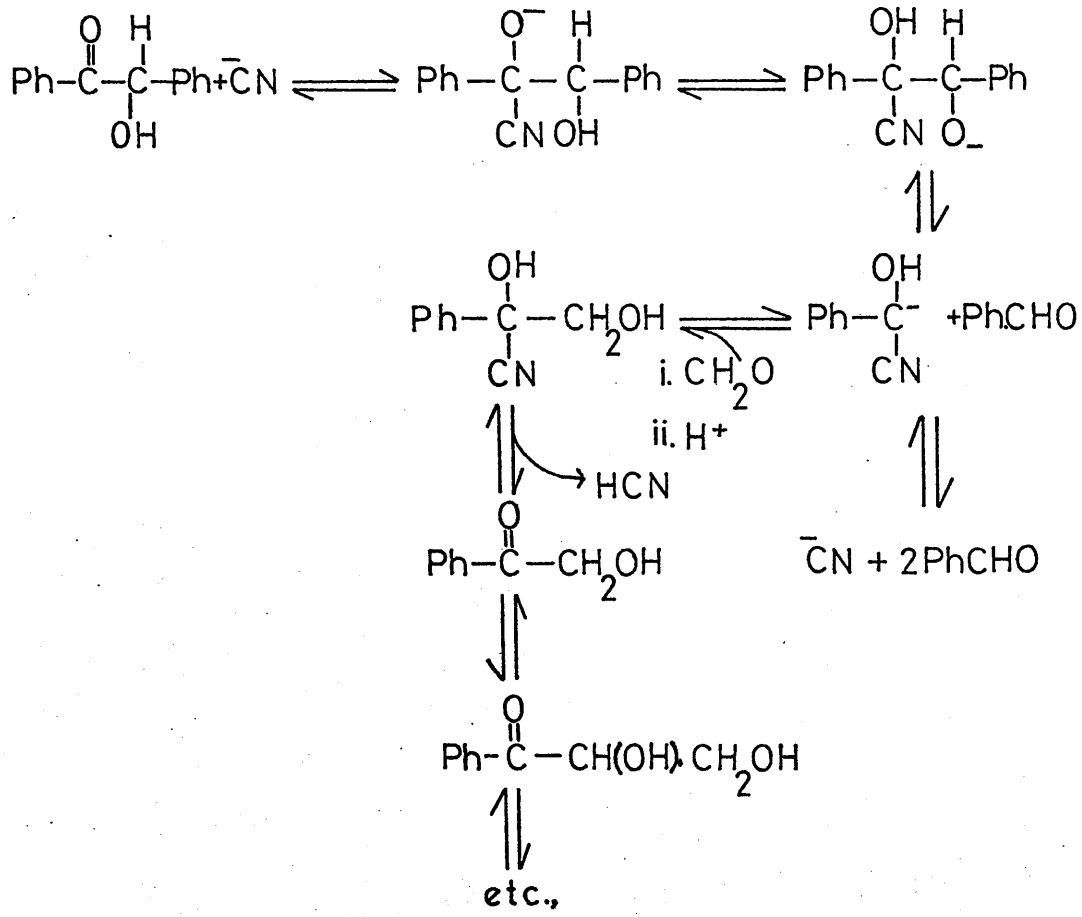
Fig.XIIb

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 42.5mg benzoin + 13mg. potassium cyanide (•)





POSSIBLE ROLE FOR BENZOIN IN INDUCING THE FORMOSE REACTION IN THE PRESENCE OF CYANIDE.



Accelerators of the Alumina - Catalysed Formose Reaction

In Chapter 1 we saw how an ene-1,2-diol might participate actively in the cycle of aldol condensations (Breslow), or how it might actively enhance the formation of the formaldehyde carbanion (Runge and Mayer), or how it might have a passive role in promotion the primary reaction by acting as a ligand to the cation and so make the formose complex more likely than the Cannizzaro complex (Pfeil). The Runge and Mayer mechanism for the first stage of the formose reaction is not helped by Kuzin's observation that ethoxy-acetaldehyde was an accelerator.[49]

The distinction between the active and the passive roles is not possible with sugars themselves, but model compounds can be used.

Benzoin and Related Compounds.

The way in which benzoin accelerates the formose reaction is not immediately obvious, if an active participation of the molecule is sought. Langenbeck's mechanism provides for the "activation" of a formaldehyde molecule by a method that is not altogether clear. His involvement of benzoyl carbinol is open to another interpretation.

In order to confirm the previous observation, benzoin was added to the alumina-formaldehyde system. It was found to cause a slight reduction of the lag period (Fig.XIIa). The rates of uptake of formaldehyde are similar in the second stage, irrespective of the presence of benzoin. This suggests that benzoin provides glycolaldehyde, which then causes autocatalysis. As benzoin cannot form glycolaldehyde by aldol condensations with formaldehyde, another mechanism is required. It is possible that the benzoin could cleave (by

GRAPHS OF FORMALDEHYDE CONCENTRATION

AGAINST TIME

Fig.XIIc

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 21mg benzaldehyde

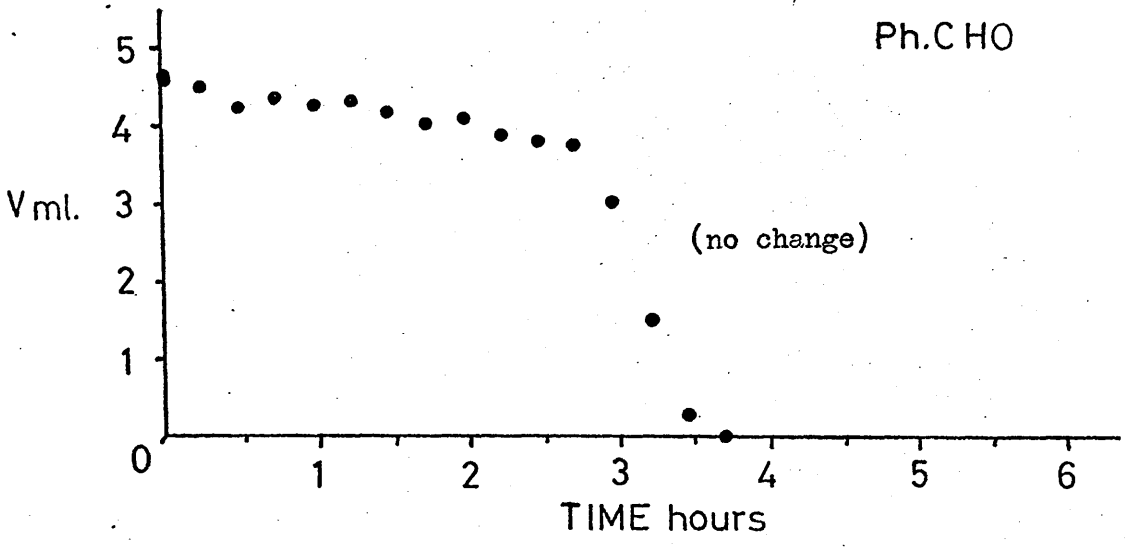
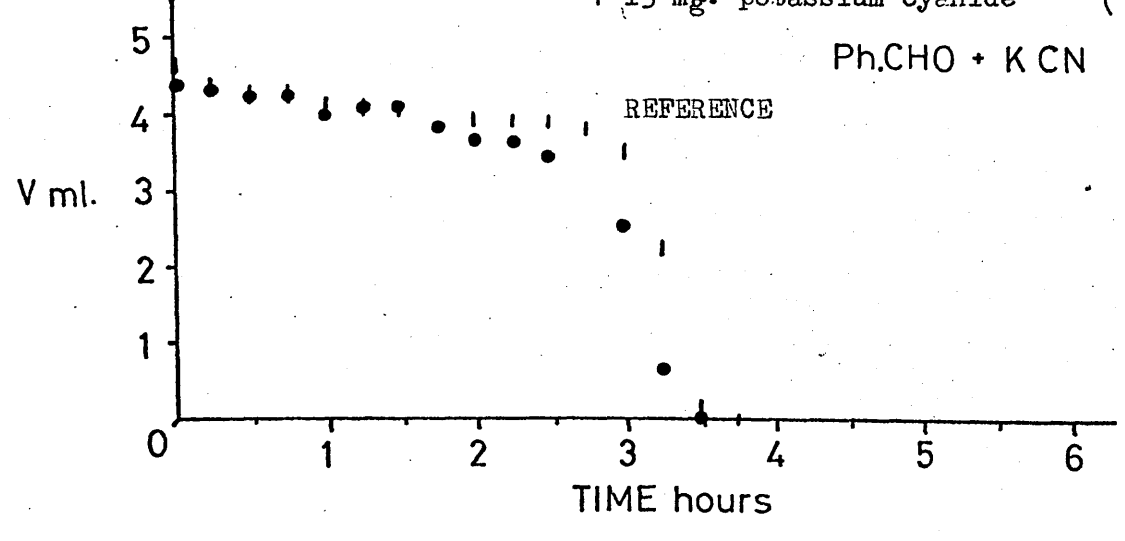


Fig.XIId

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 21mg benzaldehyde

+ 13 mg. potassium cyanide (•)



the retro-benzoin condensation) to benzaldehyde which could then form benzoyl carbinol, a known accelerator. Benzoyl carbinol can participate by the series of aldol reactions and isomerisations described in Chapter 1 (The Uniqueness of the Formose Reaction), which results in glycolaldehyde.

If this hypothesis is correct, we should expect cyanide to enhance the accelerating ability of benzoin by enabling the retro-benzoin reaction to occur more easily. The formation of benzoyl carbinol should also be easier, if the benzaldehyde cyanohydrin carbanion has not decomposed to benzaldehyde and cyanide at a more rapid rate than it condenses with formaldehyde. This is observed (Fig.XIIb). (Cyanide alone does not accelerate the formose reaction (Fig.XI).) The reaction scheme involving the cyanide ion is given opposite. In the absence of cyanide the hydroxide ion would participate.

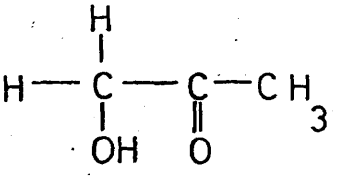
Benzaldehyde, and benzaldehyde plus cyanide (added independently) cause no and a very slight acceleration, respectively (Fig.XIIc,d). This helps to confirm the suggestion that the formation of the carbanion is rate determining. (In the benzoin condensation itself, which is reversible, this step is one of the two rate controlling processes: the other is the formation of the carbon-carbon bond.[145]) The addition of benzaldehyde and cyanide together would be expected to have a greater effect than their independent addition. These results also suggest that the aldehydic hydrogen atom is more acidic in benzaldehyde cyanohydrin than in formaldehyde cyanohydrin, as we would expect.

These results support the active participation of benzaldehyde, rather than the passive.

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIIe

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 15mg. acetol (•)



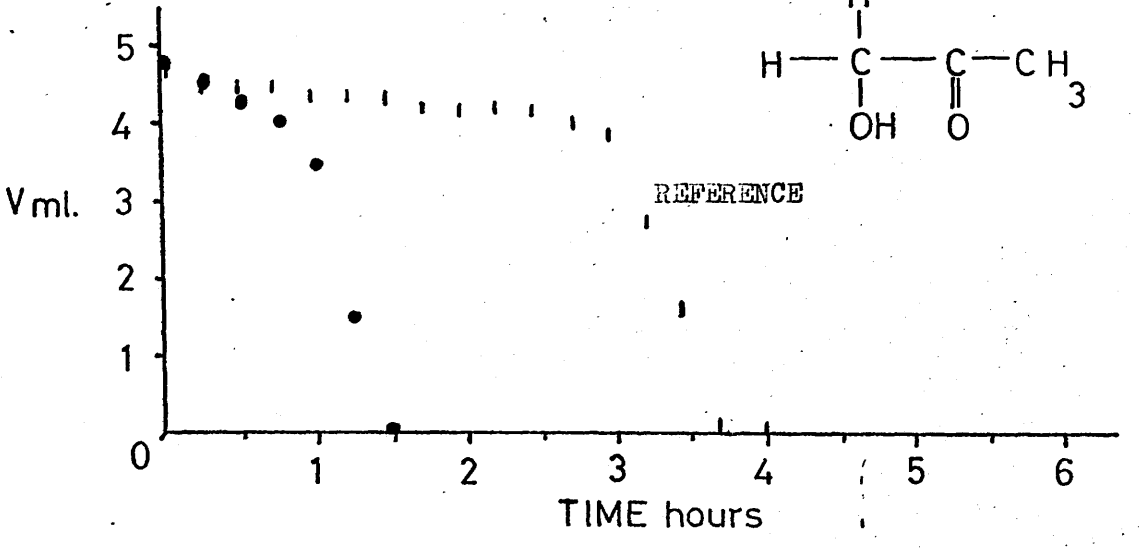
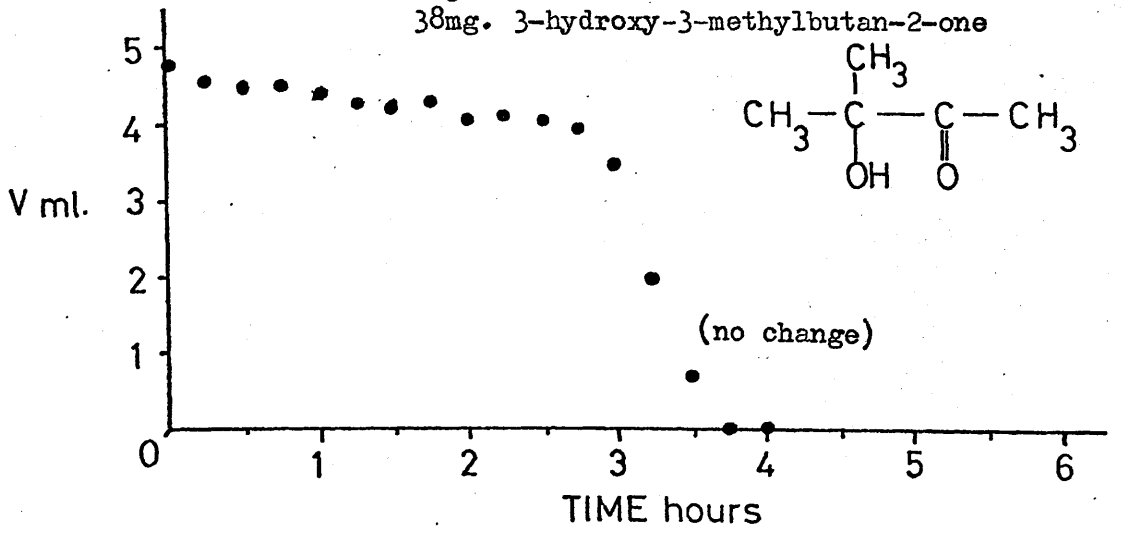


Fig. XIIf

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
38mg. 3-hydroxy-3-methylbutan-2-one



GRAPHS OF FORMALDEHYDE CONCENTRATION

AGAINST TIME

Fig. XIIg

30g. n-Al_2O_3 + 150ml. 0.13M aq.PFA. +
35mg. 4-hydroxybutan-2-one

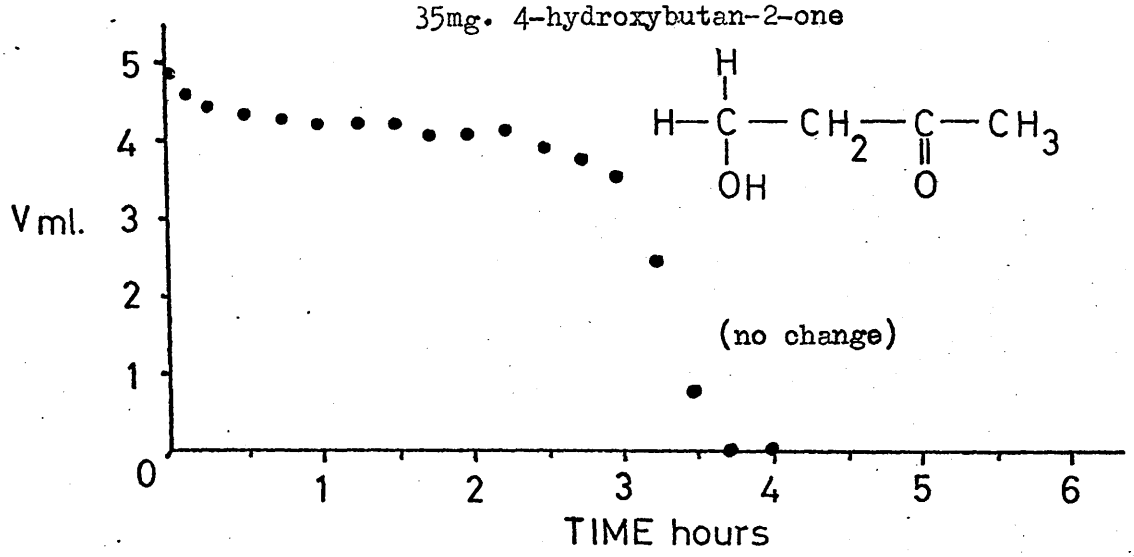
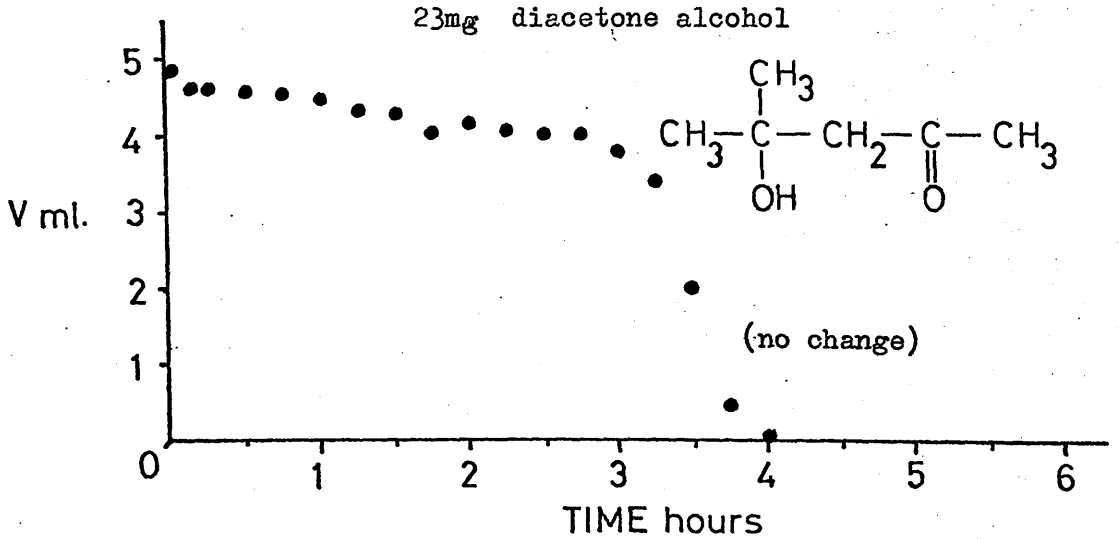


Fig. XII h

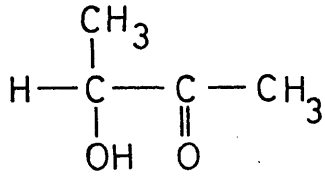
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
23mg diacetone alcohol



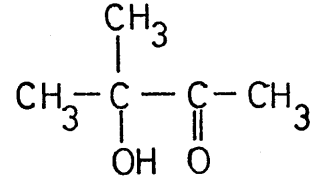
Further Studies on Accelerators.

In order to make further distinctions between the two roles of the accelerator, a series of compounds bearing different acyloin or related groups was examined.

Acetol (1-hydroxypropan-2-one) and 3-hydroxy-3-methylbutan-2-one are both α-hydroxyketones: the latter bears two methyl groups on the α-carbon. Only the former can form an enediol with the double bond between the two hydroxyls. (The latter can form an enediol with an exo double bond.) Only the former can actively participate in a cycle of aldol condensations resulting in glycolaldehyde: the mechanism is similar to that for benzoyl carbinol. Acetol only is an accelerator (Fig.XIIe,f). Again this supports the active role.

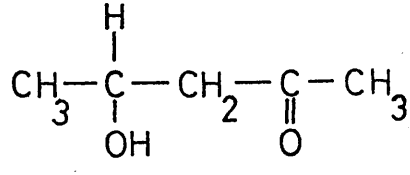


XIIe

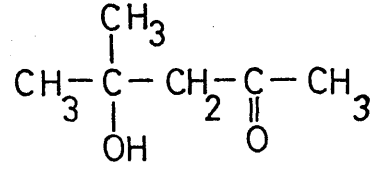


XIIf

Insertion of a methylene group between the acyloin carbons results in 4-hydroxybutan-2-one and diacetone alcohol respectively. Neither of these causes an acceleration of the formose reaction (Fig.XIIg,h). Neither can participate in an active mechanism, but both could have had a passive role.



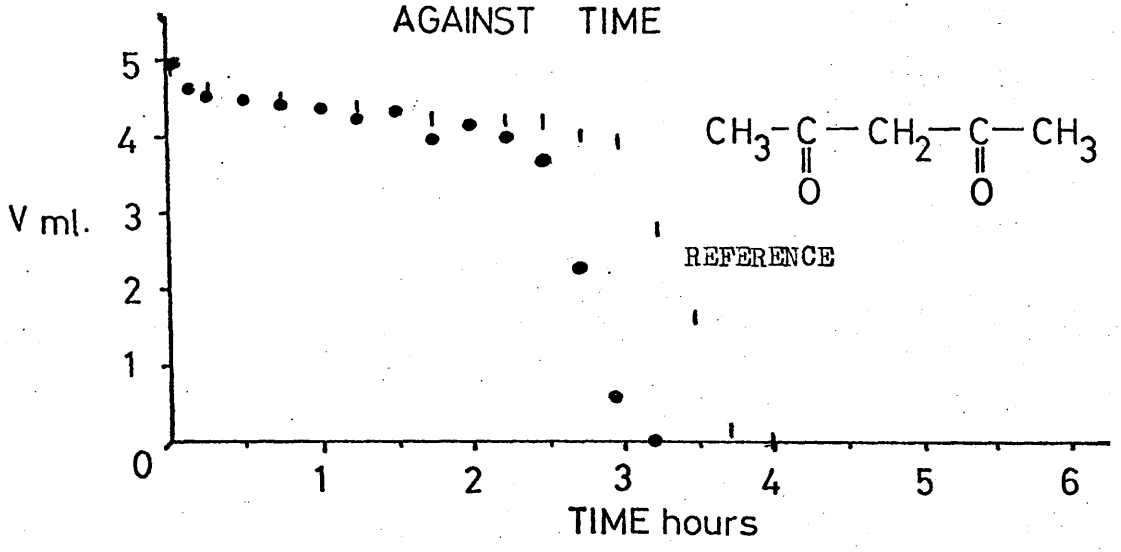
XIIg



XIIh

Fig. XIIi

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 20mg. acetylacetone (●)

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIIj

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
42mg. benzil

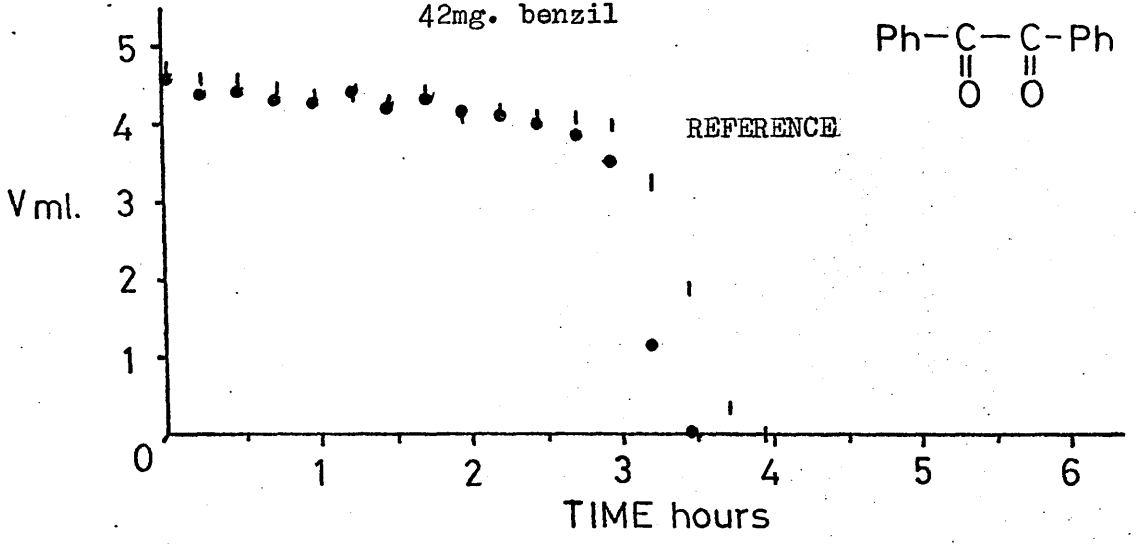
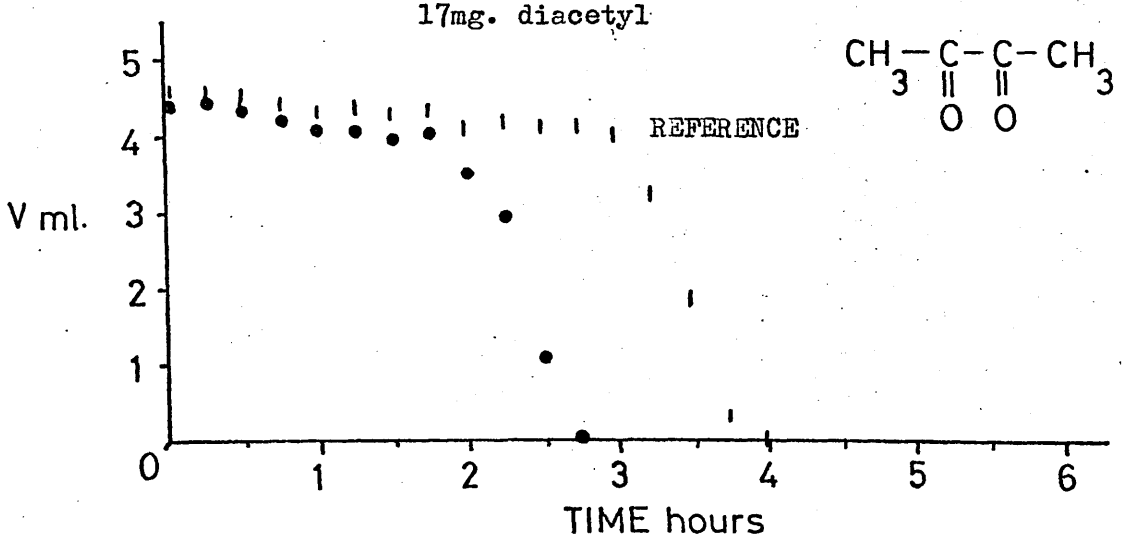
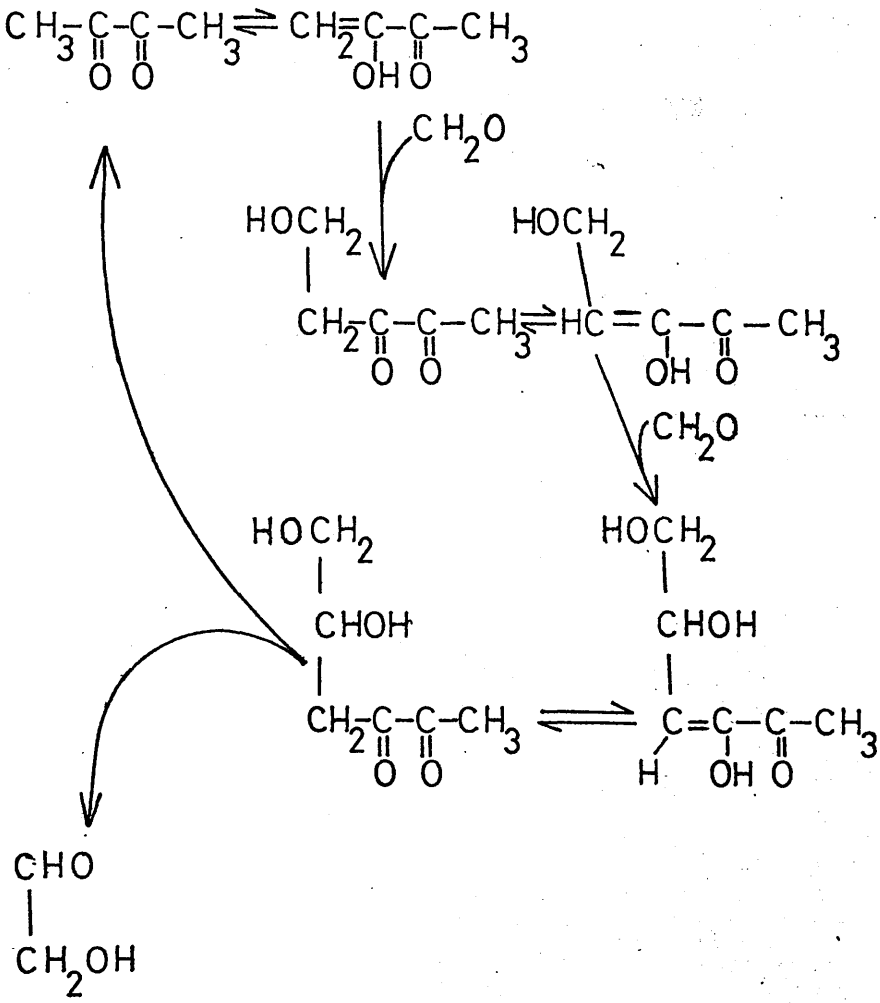


Fig. XIIk

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
17mg. diacetyl



DIACETYL MECHANISM



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIII

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
47mg. 2,3,4,6-tetra-O-methylglucose (●)

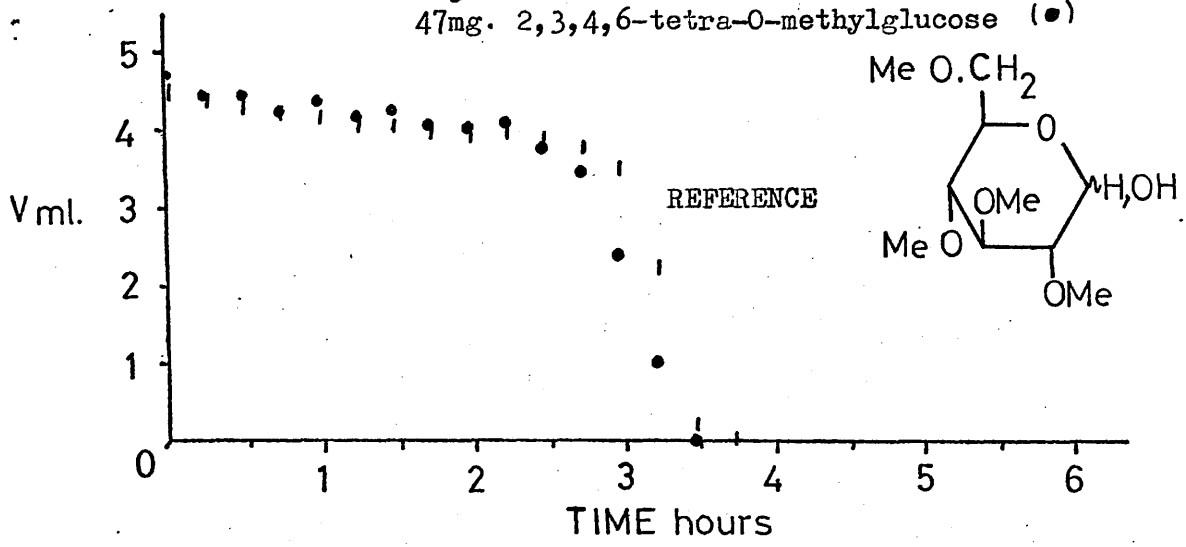
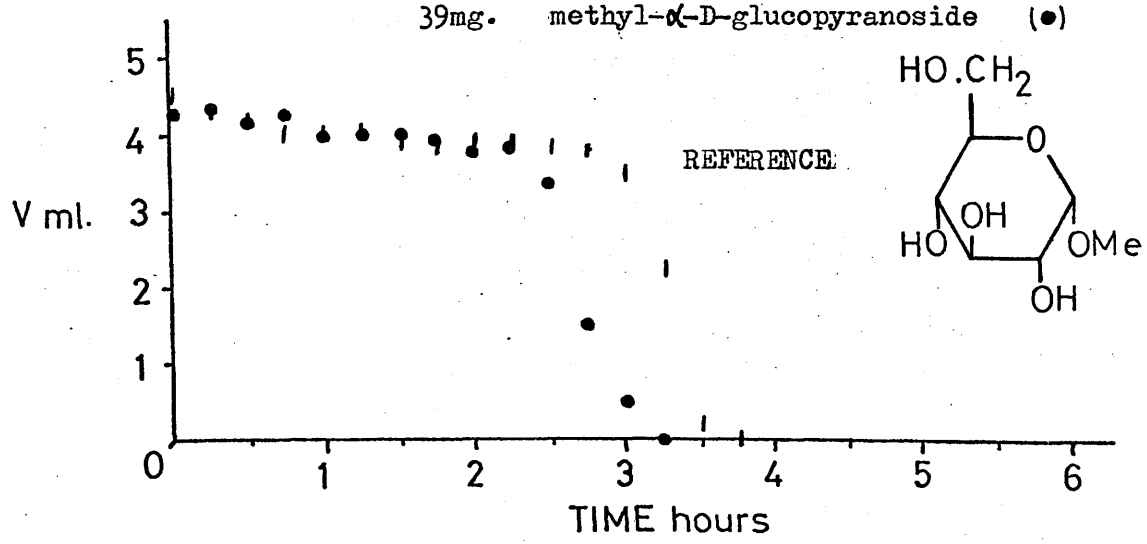
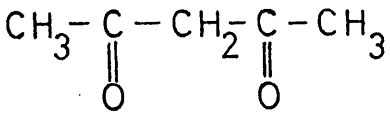


Fig. XII m

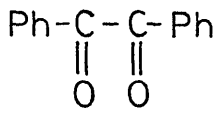
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +
39mg. methyl-α-D-glucopyranoside (●)



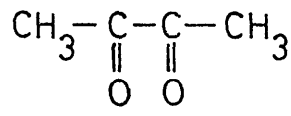
The well-known ligand acetylacetone has no effect on the rate of the reaction (Fig.XIIi), but compounds in which the carbonyl groups are in an α-positon have (Fig.XIIj,k).



XIIi



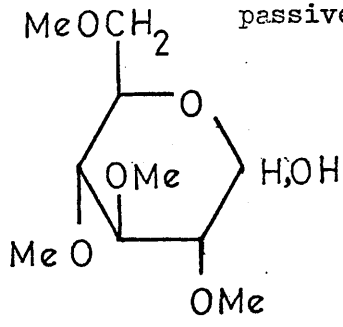
XIIj



XIIk

Diacetyl at first sight would seem to be behaving as a ligand, but may have an active role (opposite). Benzil on the other hand, can support only the passive role: its acceleration is slight. We shall return to diacetyl later.

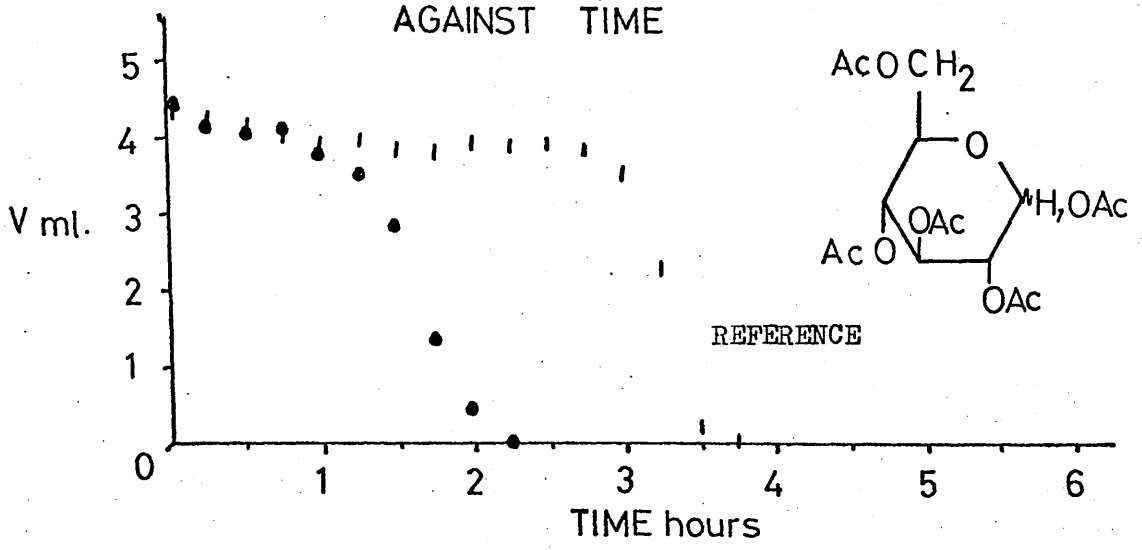
A number of sugar derivatives were next examined. The replacement of hydrogens on the 2-, 3-, 4- and 6- oxygens of glucose by methyl groups allows the formation of only a blocked enediol. A very small acceleration was found (Fig.XIIl). This supports Kuzin's finding that ethoxyacetaldehyde increased the rate, but the reason for this remains obscure, unless the compound has a passive role.



XIIl

Fig. XIIn

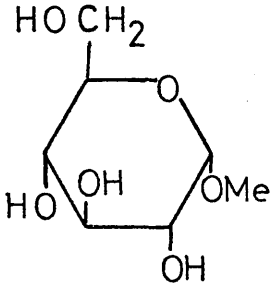
GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



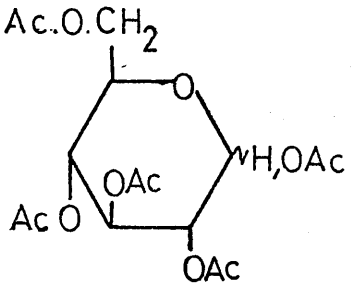
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 78mg. penta-O-acetylglucose (•)

Methylation of the 1-hydroxyl in glucose prevents enolisation, and no acceleration would be expected. A slight acceleration was found (Fig.XIIm), which is not easily explained on the basis of the purely active type of mechanism.

Penta-O-acetylglucose was added to alumina and formaldehyde and caused a large acceleration (Fig.XIIn).* This may be due to hydrolysis of the 1-acetyl and the 2-acetyl groups at least.

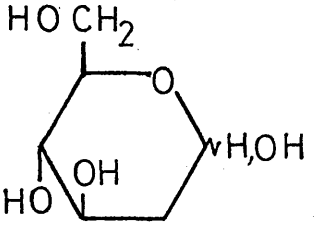


XIIm



XIIn

2-deoxyglucose cannot form an ene-1,2-diol, yet it is an efficient reducer of the induction period (Fig.XIIo). It is able to undergo retroaldolisation to acetaldehyde and aldotetrose, the latter being a known accelerator. Acetaldehyde on its own has no effect (Fig.XIIp). This shows how easily the retroaldol reaction occurs in this system.



XIIo

$CH_3.CHO$

XIIp

* Kuzin claimed that penta-O-acetylfructose was not an accelerator in the lime-catalysed system. (See Chapter 1, Accelerators.)

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIIq

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 34mg. L-ascorbic acid.

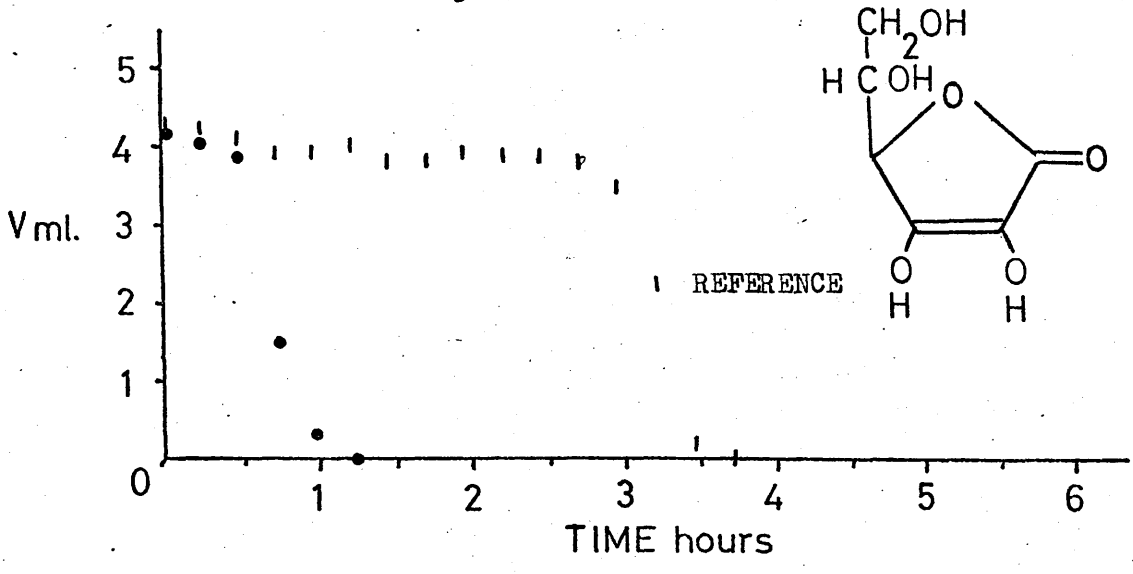
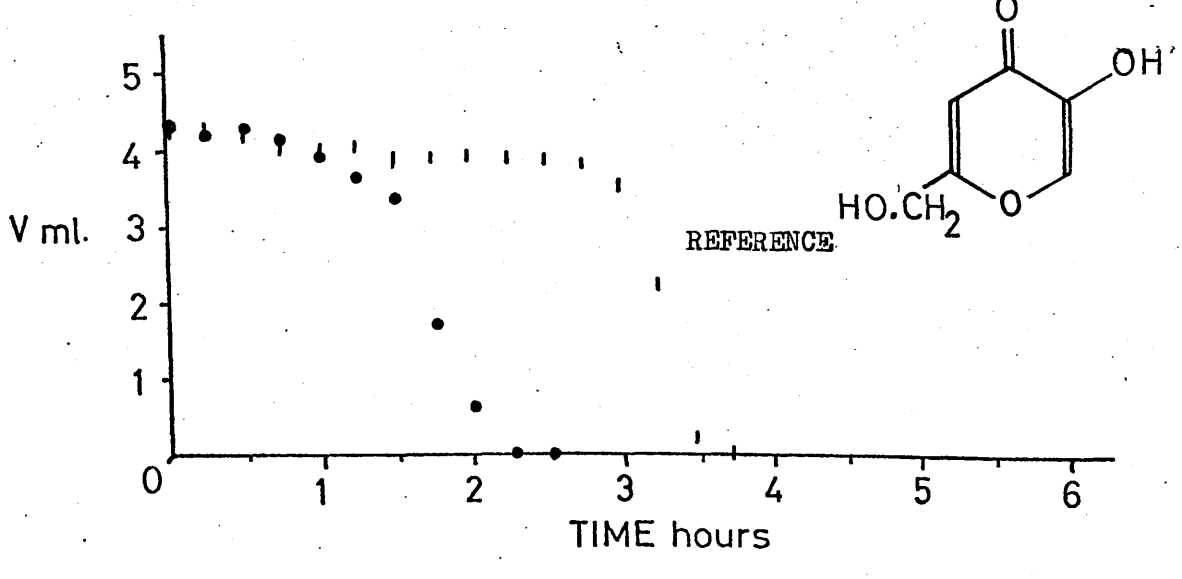
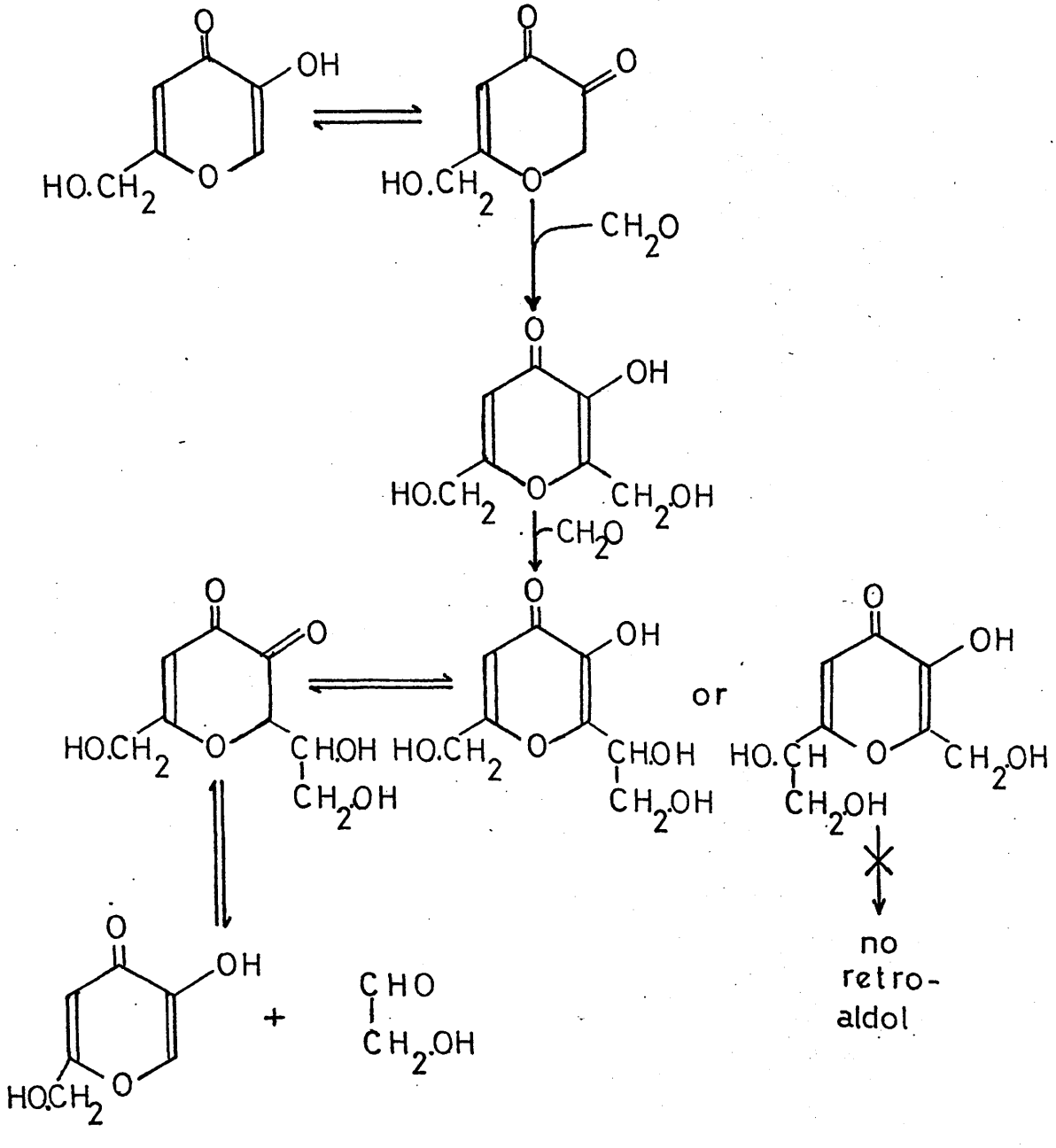


Fig. XIIr

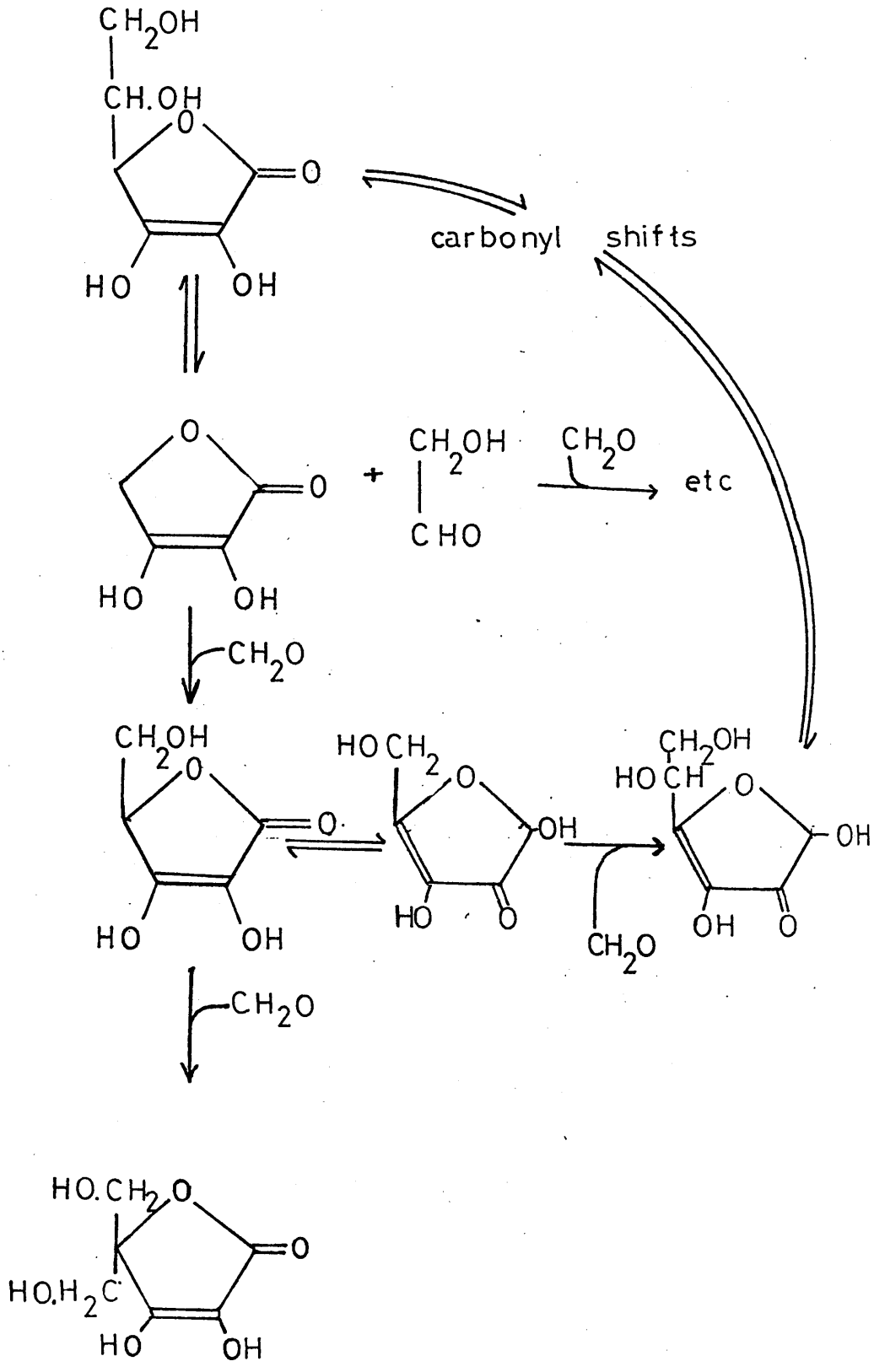
30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 28mg. kojic acid



KOJIC ACID MECHANISM

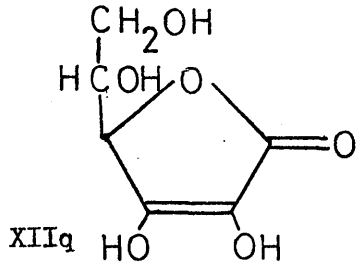


ASCORBIC ACID'S ROLE



L-Ascorbic Acid and Kojic Acid as Formose Reaction Accelerators.

The observation that L-ascorbic acid, vitamin C, could reduce the lag period of the formose reaction was made almost forty years ago (Chapter 1), and the presence of the enediol group was thought to be the source of this ability.

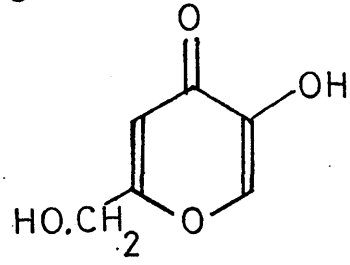


XIIq

There are, however, enediols and enediols, as we have seen, and the presence of an enediol alone is not sufficient to confer accelerating properties on the holder. Convincing mechanisms involving glycolaldehyde, etc., enediols have been written (Chapter 1, Breslow), but no mechanisms can be found for benzoin, 3-hydroxy-3-methylbutan-2-one, etc., which involve the same kind of reactions. Ascorbic acid is one of the latter class. Evidence of the combination of formaldehyde and the acid acid has been obtained,[52] but may not be relevant.

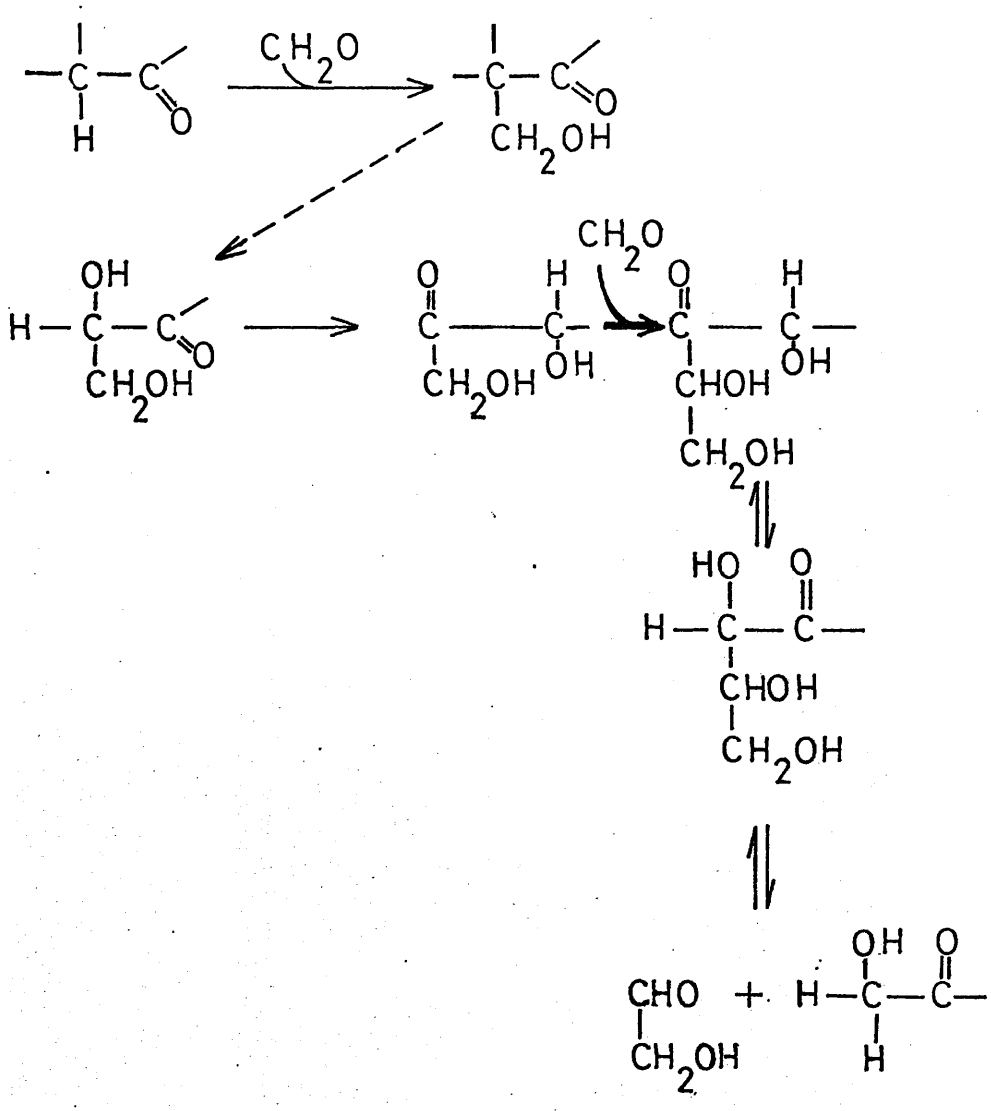
L-ascorbic acid has been confirmed as a formose reaction accelerator (Fig.XIIq), and a simple way of explaining its accelerating ability found (opposite) — the molecule can undergo retroaldolisation to glycolaldehyde.

Kojic acid possesses an acyloin group. Its accelerating properties (Fig.XIIr) may be explained by the series of reactions on the facing page.



XIIr

GLYCOLALDEHYDE TYPE OF ACCELERATOR

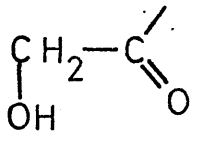


The ways in which kojic acid and glycolaldehyde are involved as accelerators are different. What are the minimum requirements of each type of compound?

a. The Glycolaldehyde Type.

The stages in the uptake of formaldehyde by aldol reactions are depicted on the opposite page: this is a repetition of the Breslow mechanism. Formaldehyde is first compounded with the enediol: this requires an active hydrogen α to the carbonyl. This formaldehyde "molecule" must then be activated to further aldol condensation. This is done by isomerisation of the carbonyl group. Once the second formaldehyde molecule has been accepted, the 2-3 carbon bond must be broken. This can only be done if the carbonyl shifts to its original position. We now have two moles of glycolaldehyde, one the accelerator, the other from formaldehyde.

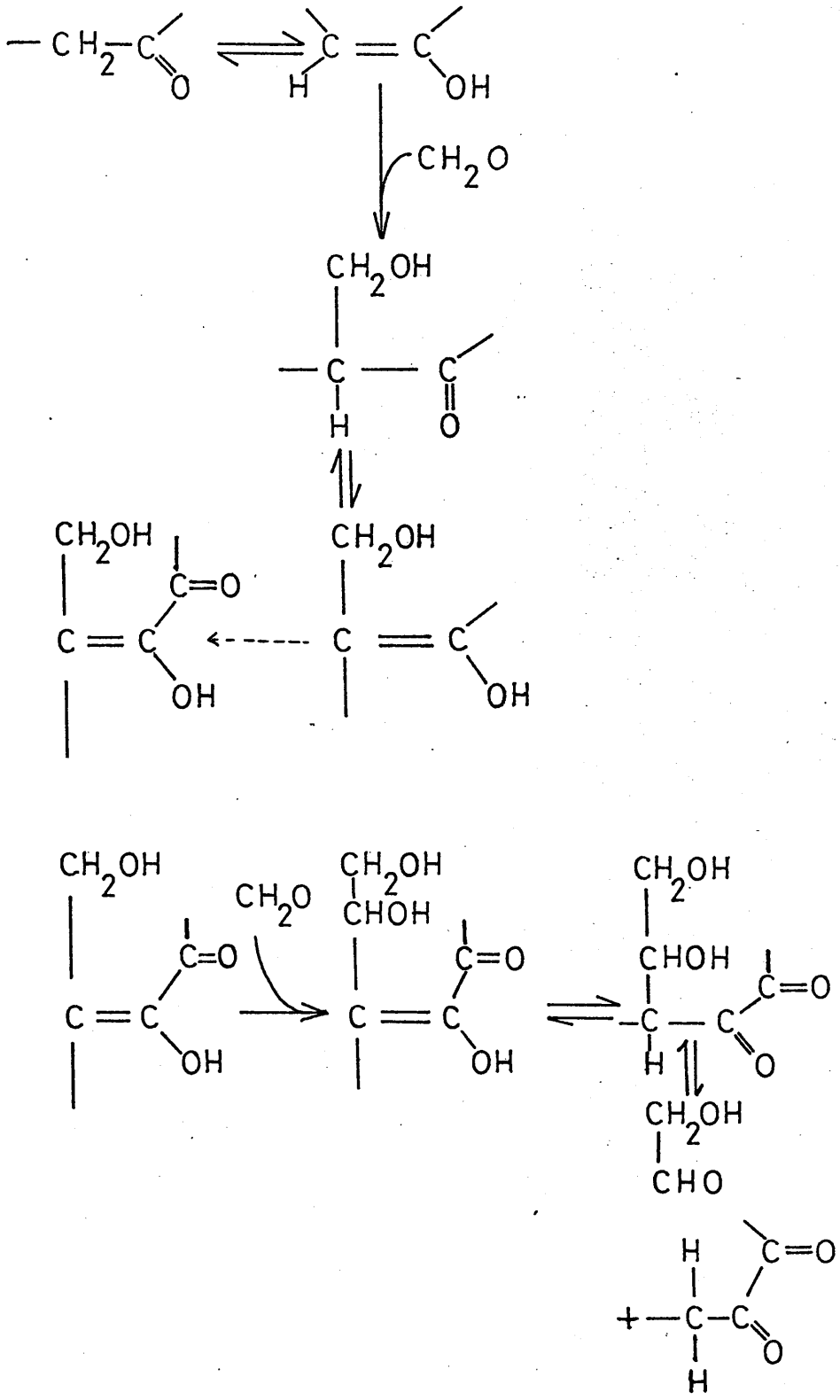
The minimum requirements of this system are therefore:



A methylenic carbon is necessary, because one hydrogen is needed for aldol condensation and one to enable the carbonyl to move.

The presence of glyceraldehyde and 1,3-dihydroxyacetone in this mechanism is obvious, as is the presence of aldo- and keto-tetrose. Compounds possessing the hydroxymethyl ketone group range from fructose to benzoyl carbinol.

KOJIC ACID TYPE OF ACCELERATOR

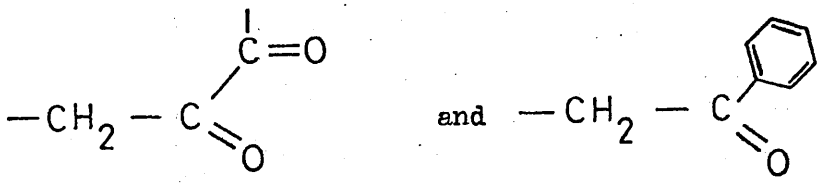


b. The Kojic Acid Type.

Let us start with the mono-substituted methyl ketone as our accelerator. (This group is present in one of the tautomeric forms of kojic acid.) The scheme is depicted opposite.

A group which can stabilise the anion formed on the carbon of the first molecule of formaldehyde which has been condensed with the accelerator is needed. (Acetaldehyde does not accelerate the formose reaction.) Such stabilisation will be provided by a carbonyl group or a phenyl ring.

The minimum requirements for this type of accelerator are therefore:



As examples, we make take kojic acid, biacetyl and benzoyl carbinol. The two ways in which benzoyl carbinol can be involved may be the reason for its excellent accelerating properties.

A Cautionary Note.

Of the two types of accelerator that we have discussed, there do not seem to be any partic ular factors which would lead us to chose one in preference to the other. We should however remember that diacetyl (and methyl glyoxal, for that matter) has been isolated as a decomposition product of sugar.[28]

Table XIII

ATTEMPTED FORMOSE REACTIONS USING DIFFERENT AMOUNTS OF BASIC-ALUMINA AND FORMALDEHYDE

MASS b-Al_2O_3	MASS CH_2O	RATIO Al_2O_3/CH_2O	MOLARITY CH_2O	FINAL COLOUR Al_2O_3	FINAL COLOUR CH_2O	FINAL CH_2O
100mg.	12mg.	1g.:120mg.	0.013	w	cl	+
100mg.	120mg.	1g.:1.2g.	0.13	w	cl	+
100mg.	1.2g.	1g.:12g.	1.3	w	cl	+
750mg.	12mg.	1g.:16mg	0.013	w	cl	+
750mg.	120mg.	1g.:160mg.	0.13	v.brn	y	0
750mg.	1.2g.	1g.:1.6g.	1.3	w	cl	+
5.0g.	12mg.	1g.:2.4mg.	0.013	w	cl	+
5.0g.	120mg.	1g.:24mg.	0.13	brn	y	0
5.0g.	1.2g.	1g.:240mg.	1.3	w	cl	+

Abbreviations:

w	white	v.brn	very brown
cl	colourless	y	yellow

GRAPHS OF FORMALDEHYDE CONCENTRATION

AGAINST TIME

Fig. XIIs

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA + 29mg 8-hydroxyquinoline

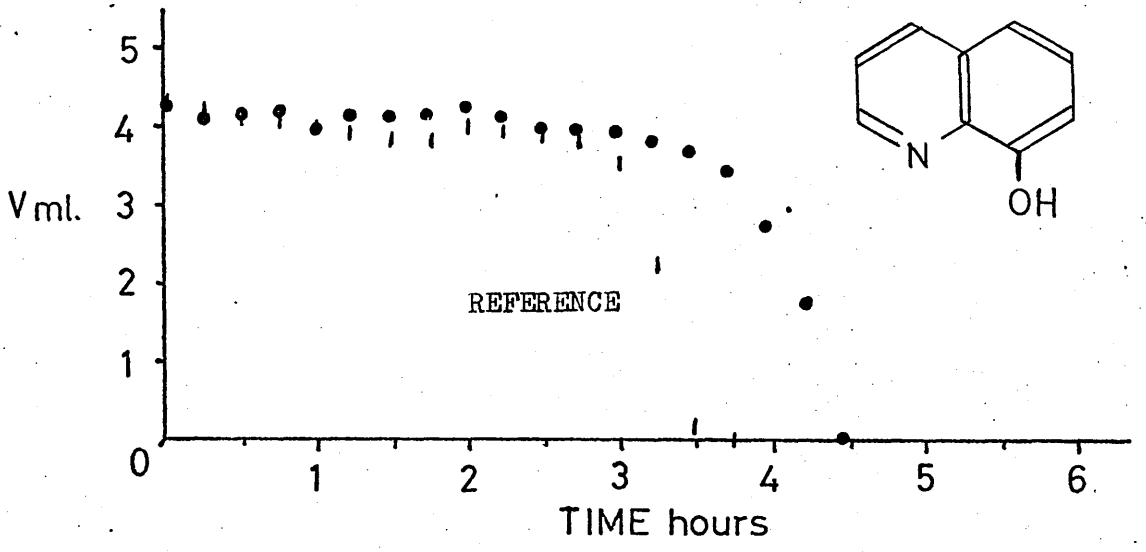
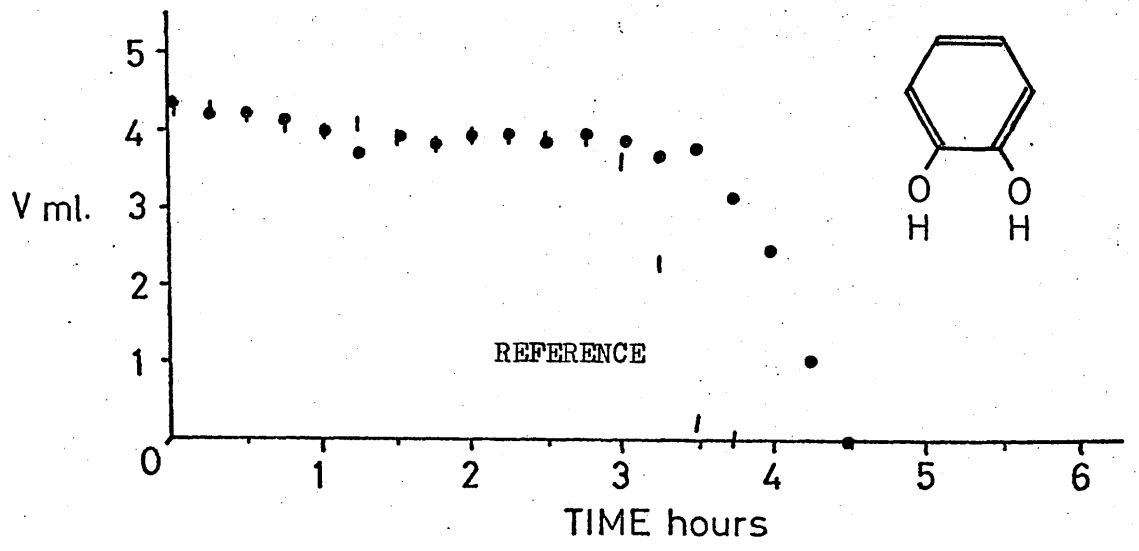


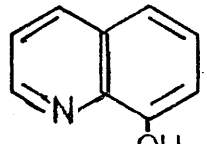
Fig. XIIt

30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA + 22mg catechol

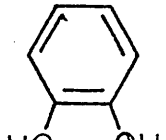


8-Hydroxyquinoline and Catechol.

Both of these compounds inhibit the formose reaction (Figs,XIIs,t).



XIIs



XIIt

Why this is so, is not clear, but two reasons can be given. Firstly, the acidity of these phenols is sufficient to reduce the pH of the medium, so that alumina must dissolve in order to attain the level at which the formose reaction may begin. The other reason could be that these compounds co-ordinate to the cations so well that entry of formaldehyde is effectively stopped until more sites are available from alumina hydration.

Further investigations of this system were not attempted.

Some Aspects of the Alumina-Catalysed Formose Reaction.

In the standard system which has been used for the formose reaction, the amount of the alumina (30g.) greatly exceeds the amount of formaldehyde (600mg.). Can the reaction be catalysed with less alumina, and what effect will the formaldehyde concentration have?

Various amounts of alumina and formaldehyde were heated together for five hours. Basic alumina was used, so that more favourable conditions were obtained, than with neutral alumina. At the end of this time the formaldehyde was estimated qualitatively, and the colours of the alumina and the solution noted. The observations are recorded in Table XIII.

The presence of sugar was indicated by a yellowing of the solution and the alumina, and the absence of formaldehyde. (These observations were also made in experiment Ia, where paper chromatographic analysis of the products, indicated the formation of compounds closely resembling sugars.) In the other experiments of this Chapter, where the formaldehyde concentration fell rapidly, the colouration of the alumina and solution were also noted.)

Only in two cases were sugars formed. In the remainder most of the formaldehyde remained after five hours, and the alumina remained white and the solution colourless.

These results strongly suggest that the reaction has been heterogenously catalysed.

i. Location of the Primary Product.

If the reaction is heterogenously catalysed, we would expect that there would be some adsorption of the accelerating species on the surface of the alumina. In order to find if this was so,

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIVa,b

150ml. 0.13M aq. PFA. + a. 100mg "used" Al_2O_3 (•)
b. 1.0g. "used" Al_2O_3 (o)

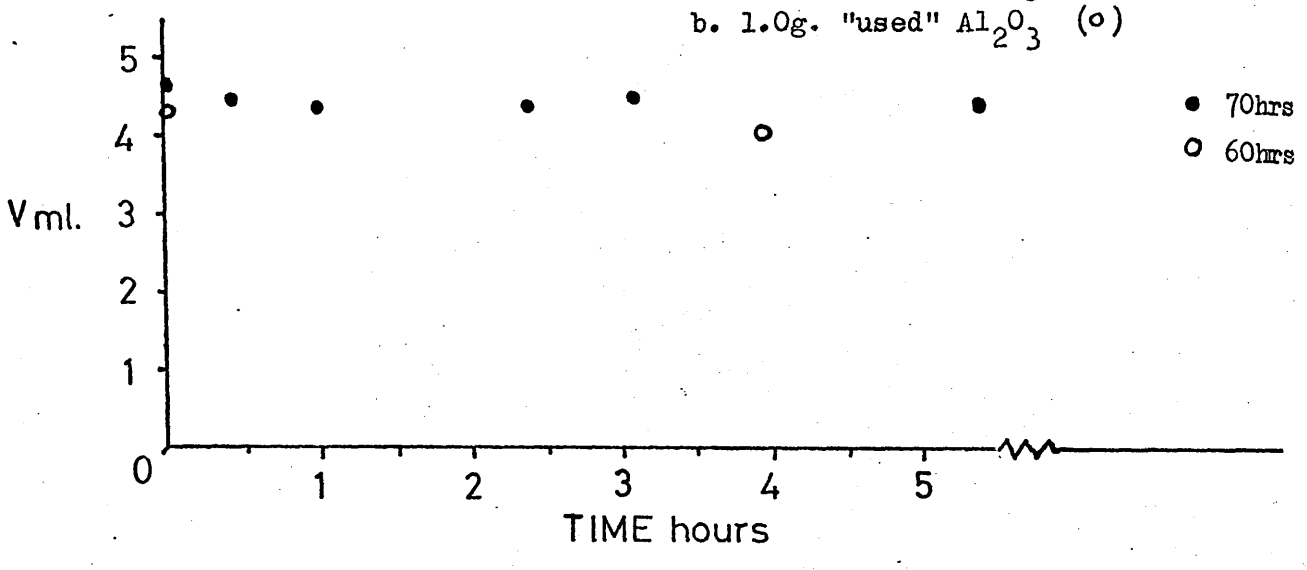
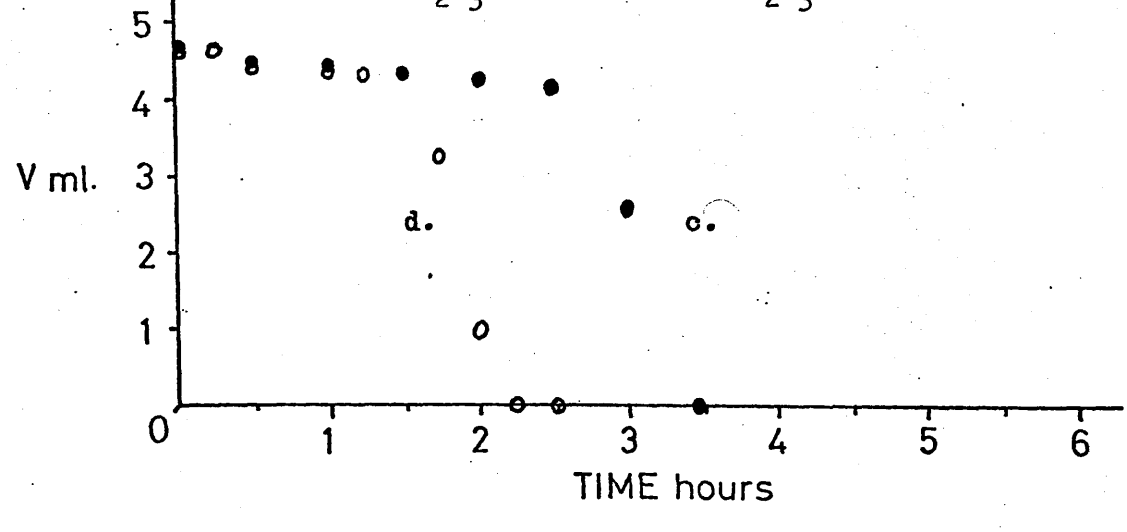


Fig. XIVc,d

c. 29.9g. n-Al_2O_3 + 100mg "used" Al_2O_3 + 150ml. 0.13M aq. PFA. (•)
d. 29g. n-Al_2O_3 + 1 g. "used" Al_2O_3 + 150ml. 0.13M aq. PFA. (o)



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XV a b c

a. 30g. n-Al_2O_3 + 150ml.0.13M aq. PFA. Refluxed until end of lag period. Phases separated and fresh reagent added.
b. "old" solution + n-Al_2O_3 c. "old" Al_2O_3 + 0.13M aq. PFA

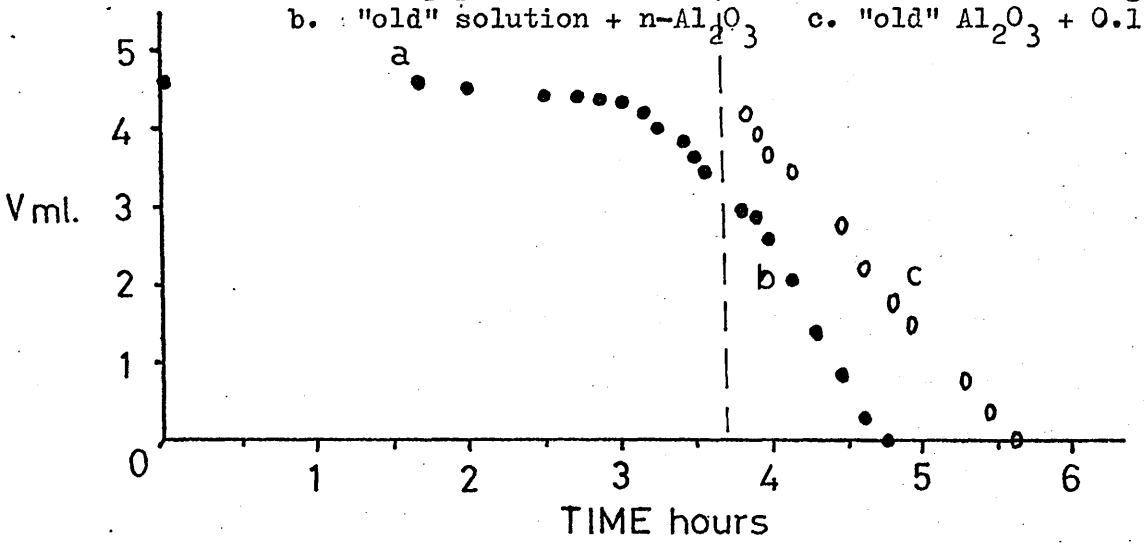
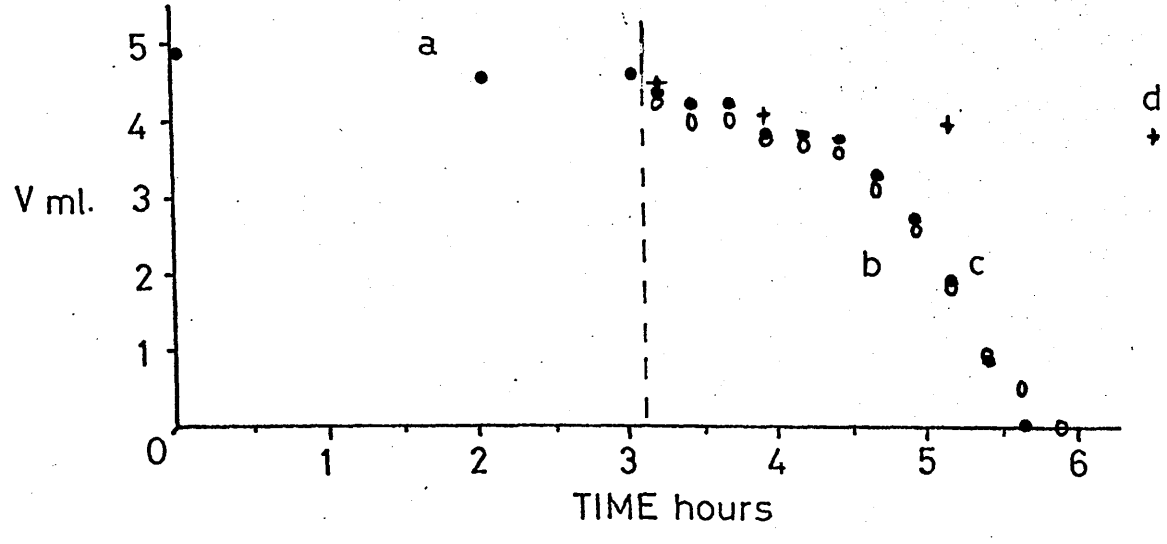


Fig. XVI a b c d

a,b,c as above.
d "old" solution only.



two portions (1.0g. and 100mg.) of alumina taken from a flask after a formose reaction lasting five hours and well washed with water, were examined for their accelerating ability on their own and in the presence of sufficient fresh neutral alumina to make 30g. total (Fig.XIVa-d). This yellow "used" alumina did not catalyse the formose reaction on its own, but an acceleration was noted when the fresh alumina was present. It seems that some accelerating species is quite firmly adsorbed onto the alumina. The necessity for 30g. total alumina may be due the pH of the solution having been insufficient for the formose reaction (see XXIa-c), or the necessity of a larger number of sites on the alumina before the formose reaction can take place. (A high formaldehyde: catalyst ratio favours the Cannizzaro reaction: Chapter 1.) By a comparison of the extent of acceleration caused by different amounts of glycolaldehyde (experiments VIIIa-c, IXa-d) we may say that not more than 1mg. accelerator (measured as glycolaldehyde) is adsorbed on 1.0g. alumina.

Is the primary species, formed at the end of the lag period liberated to the solution, or does it remain firmly attached to the alumina? Soon after the end of the induction period in a standard reaction, the reaction was stopped by chilling in ice. The solid and liquid were separated and the solid washed well with water. To each were added fresh supplies of the opposite reagent in quantities which preserved the original ratios and concentrations. The formaldehyde concentration in both fractions was then measured after they had been heated to 100°.(Fig.XV). The formaldehyde is consumed at a similar rate in both fractions. No additional induction period is observed. This implies that the accelerating species are present in the solution and adsorbed to alumina.

The experiment was repeated again, but this time the end of the lag period was anticipated (Fig.XVI). A small

Fig. XVII a,b,c

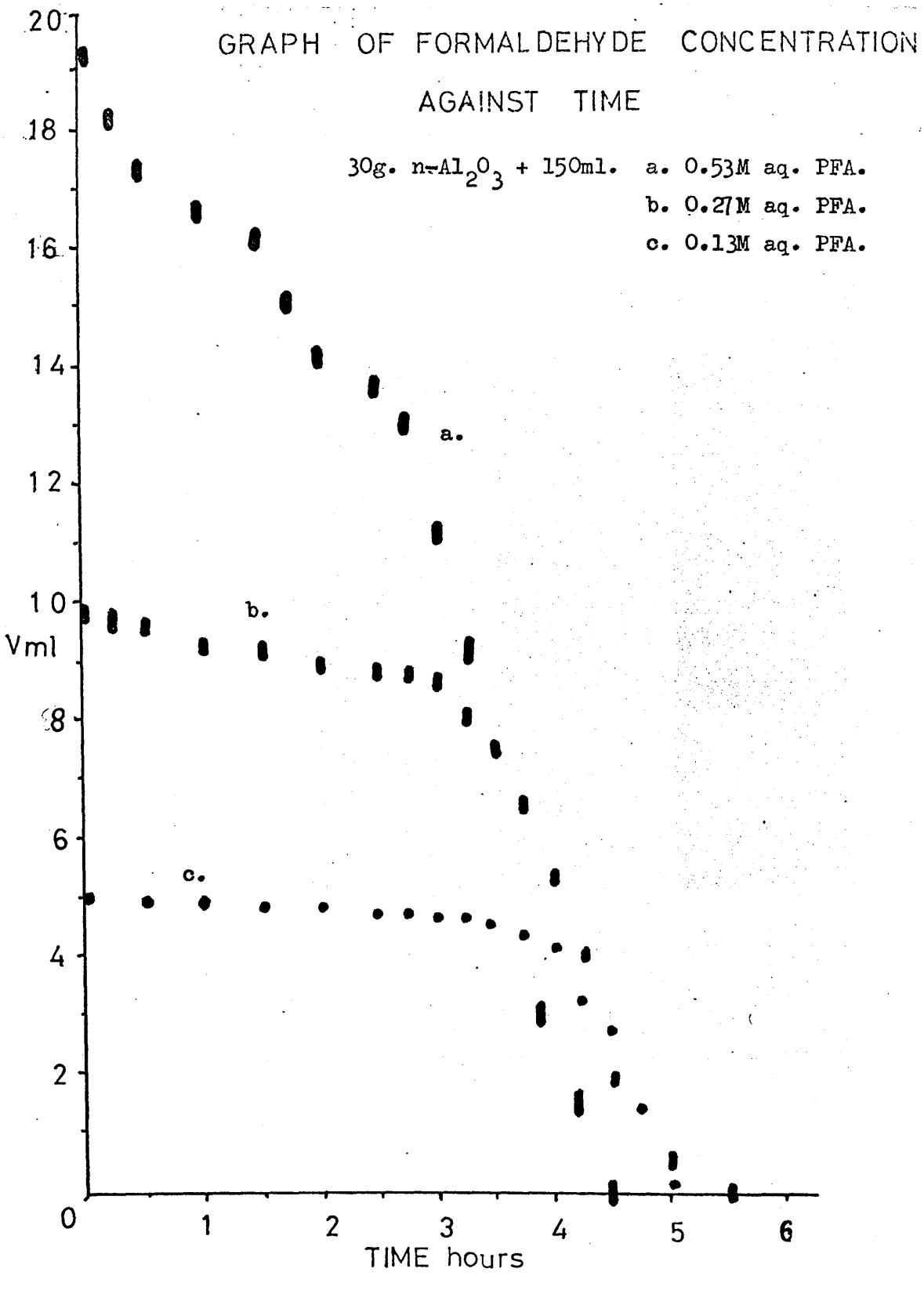
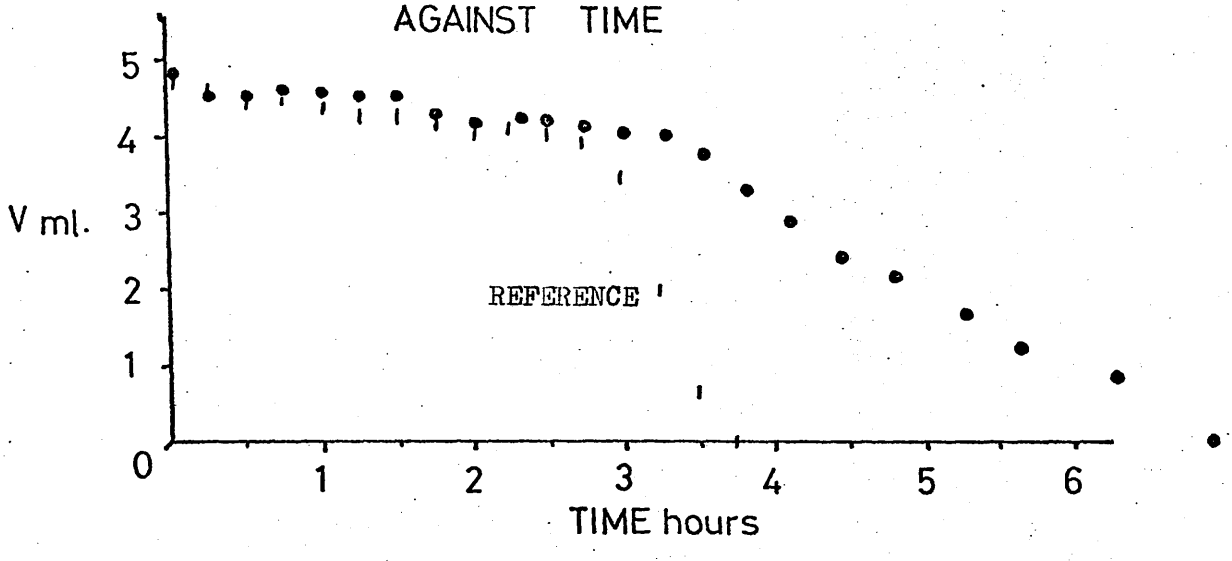

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME
30g. n-Al_2O_3 + 150ml.
a. 0.53M aq. PFA.
b. 0.27M aq. PFA.
c. 0.13M aq. PFA.
Vml
TIME hours
a.
b.
c.
0
2
4
6
8
10
12
14
16
18
20
1
2
3
4
5
6

Fig. XVII d

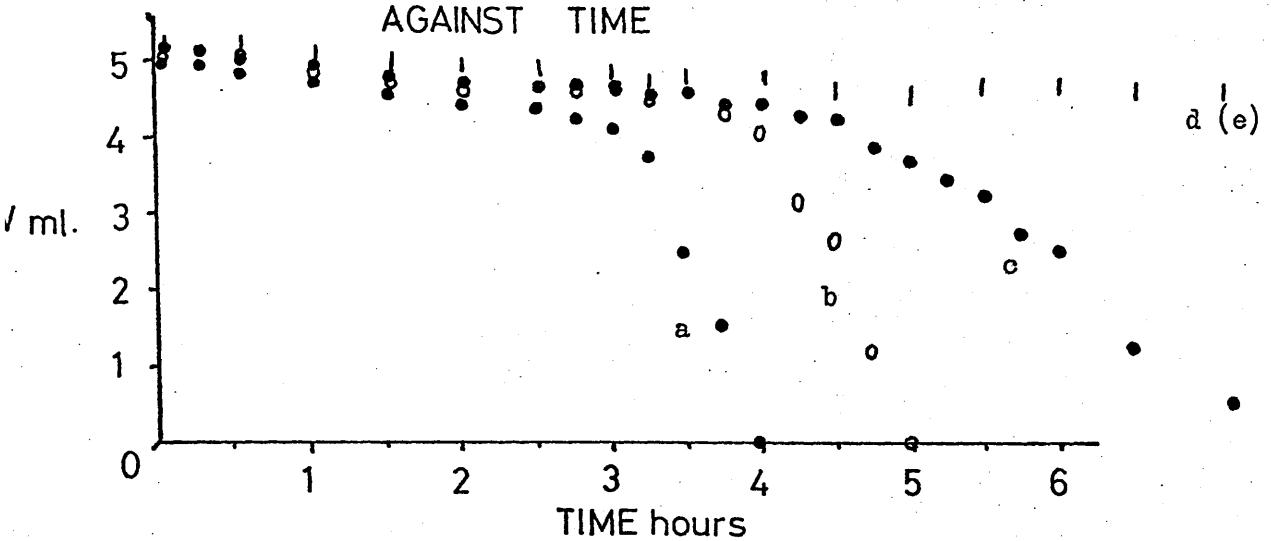
GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



30g. n-Al_2O_3 + 600ml. 0.13M aq. PFA.

Fig. XVIII

GRAPH OF FORMALDEHYDE CONCENTRATION AGAINST TIME



a. 60g. n-Al_2O_3 + 150ml. 0.13M aq.PFA

b. 30g. " " "

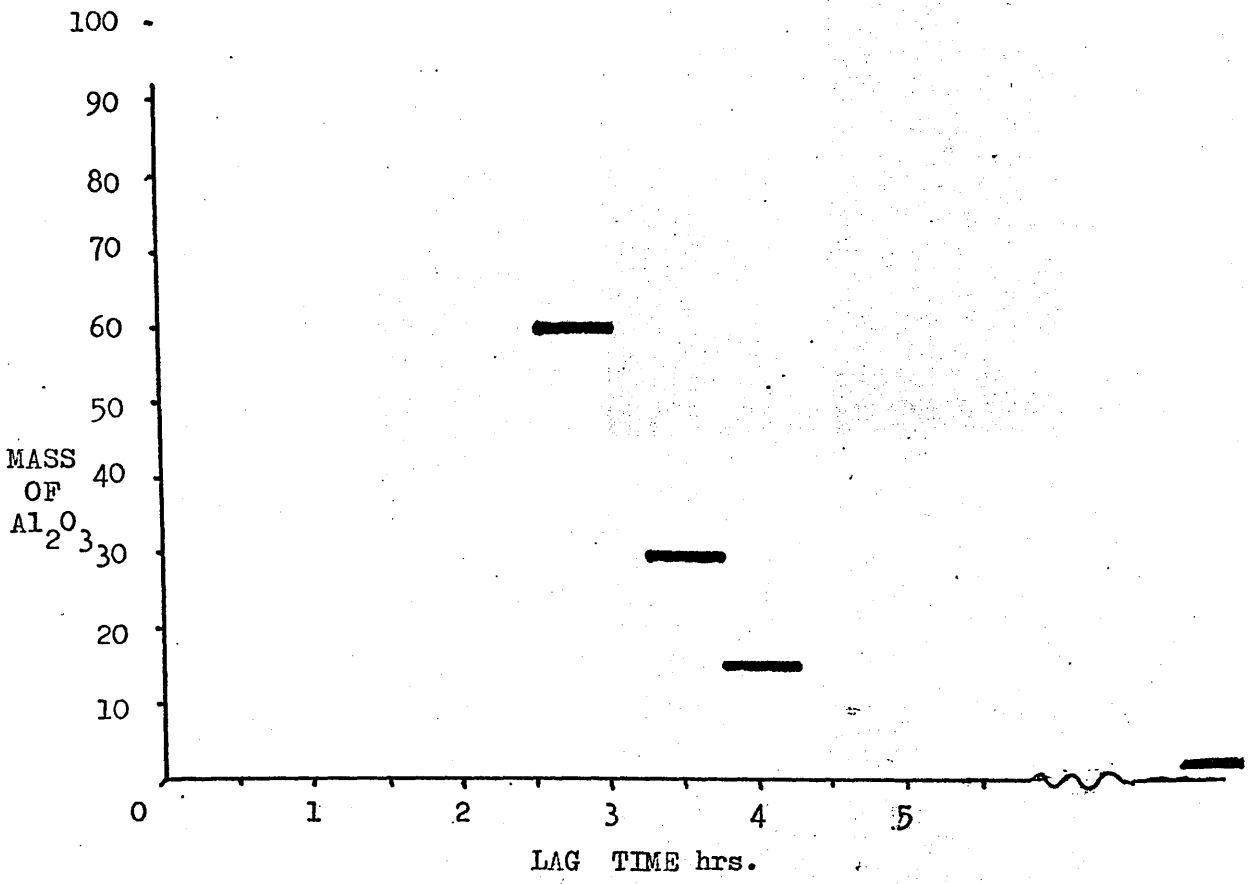
c. 15g. " " "

d. 5g. " " " 11hrs. V =1.0 22hrs. V =0.0

(e. 1g. " " " 50hrs V =2.4)

Fig XVIII'

GRAPH OF AMOUNT OF ALUMINA AGAINST LAG TIME



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIX a, b

a. 31g. n-Al_2O_3 (grade II) + 150ml. 0.13M aq. PFA (•)

b. 32g. n-Al_2O_3 (grade III) + 150ml. 0.13M aq. PFA (○)

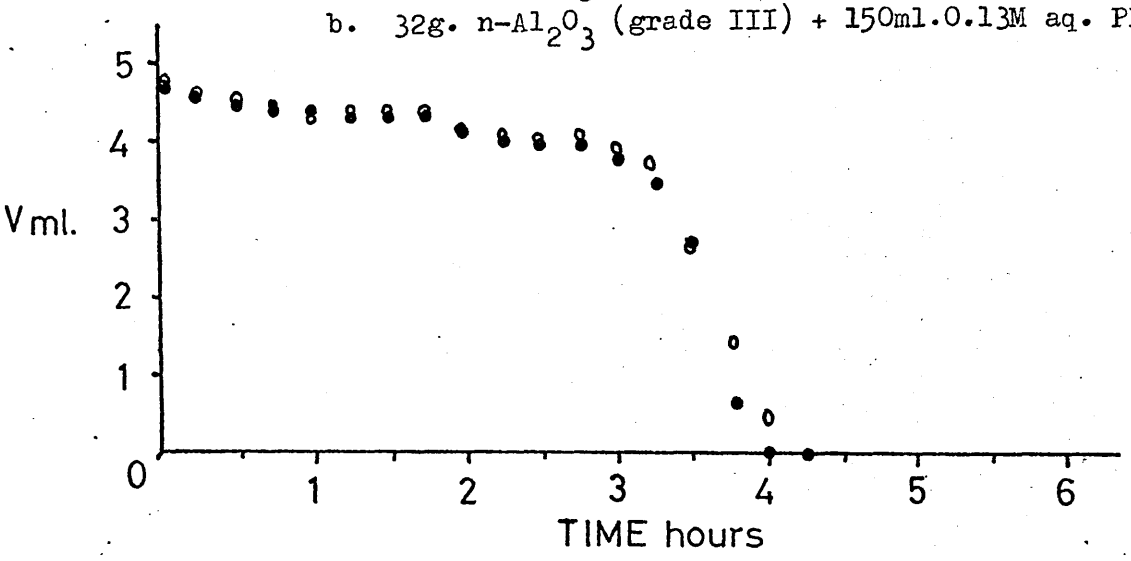
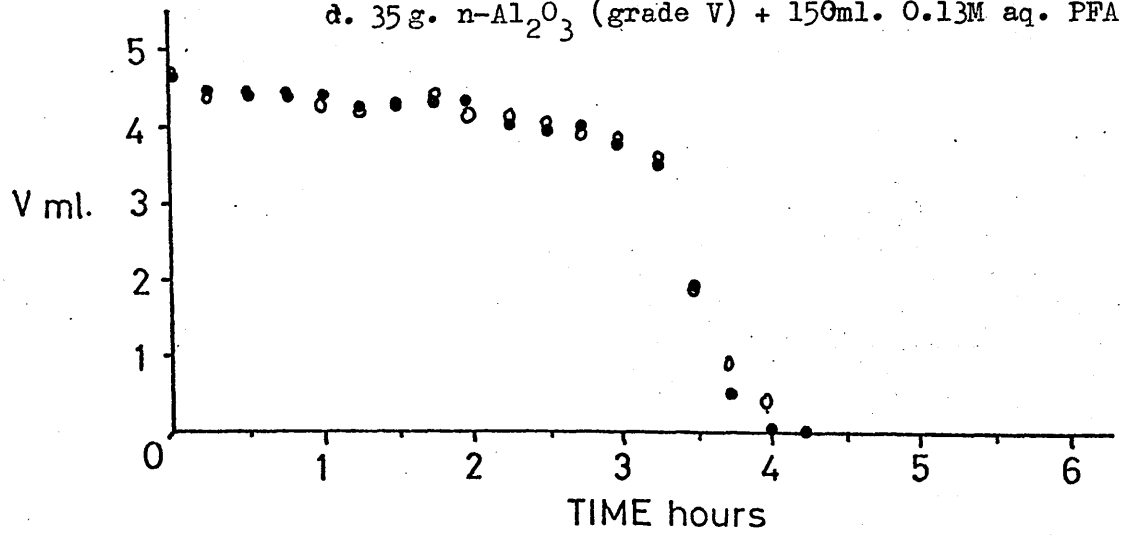


Fig. XIX c, d

c. 33⅓g. n-Al_2O_3 (grade IV) + 150ml. 0.13M aq. PFA. (•)

d. 35 g. n-Al_2O_3 (grade V) + 150ml. 0.13M aq. PFA. (○)



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XIXe

e. 37g. n-Al_2O_3(grade VI) + 150ml. 0.13M aq. PFA.

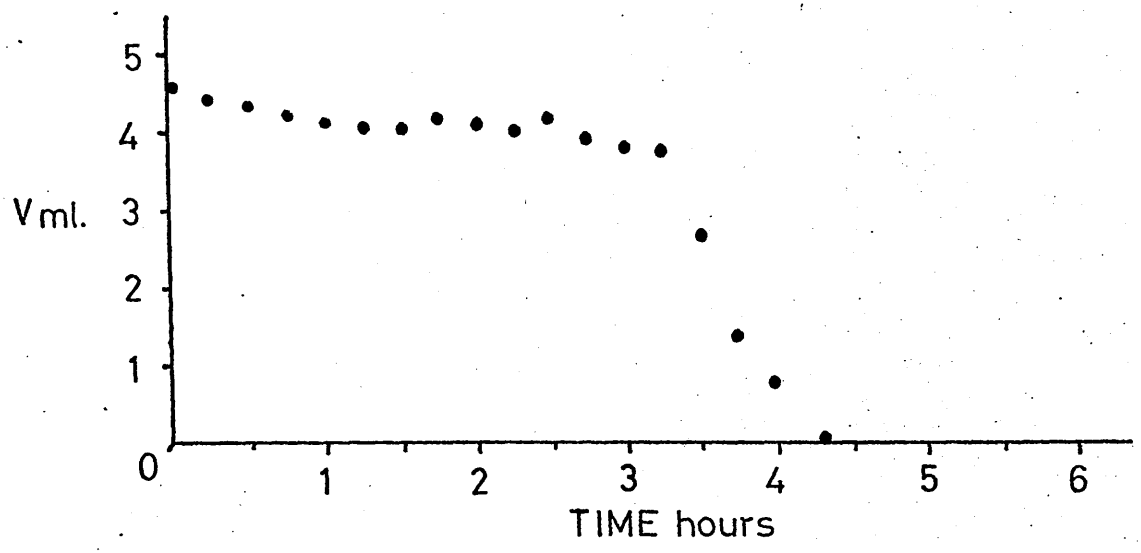
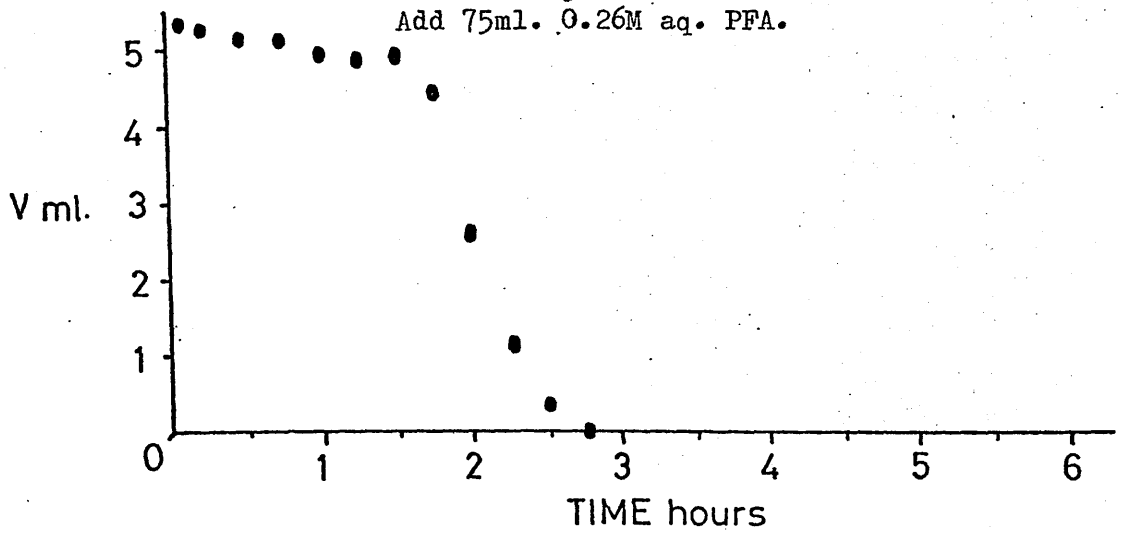


Fig. XIXf

f. 30g. n-Al_2O_3 + 75ml. H_2O reflux for 5 days.
Add 75ml. 0.26M aq. PFA.



delay is found in both systems. The presence of the additional alumina is necessary for the reaction to proceed (Fig.XVId). The simultanous rates of uptake of the formaldehyde suggests that the accelerating species are present in both fractions. A small quantity of accelerator will be formed before the apparent end of the lag time and it seems that it has been released to the solution, or at least primary complexes for the formose reaction are present in both. A small amount of alumina passed through the filter paper into the solution: this may have contained complexed accelerator, or primary complexes.

ii Alteration of the Amounts of Alumina and Formaldehyde.

An increase of the formaldehyde concentration by four-fold does not markedly increase the total time for the reaction. (Fig.XVII), and the induction period is only slightly reduced. The addition of a larger amount of formaldehyde at the same concentration as in the standard reaction (0.13M), causes a longer total time for the reaction, again in line with expectation (Fig.XVIId).

As the amount of alumina is reduced, the reaction becomes slower (Fig.XVIIIa-e). If we plot the length of the lag times against the amount of the alumina (Fig.XVIII'), a minimum lag time is suggested. Why should this be?

The alumina hydration occurs at a relatively slow rate. Perhaps this is the cause. Alumina of activity grades II to IV was examined under formose conditions (Figs.XIXa-e) and similar lag times were found. The addition of formaldehyde to 30g. neutral alumina which had been heated with water for 5 days (effectively activity grade XXV !) enables a shorter reaction time to be observed (Fig.XIXf). The induction period has been reduced, and the rate of formaldehyde uptake in the second stage has been increased.

GRAPHS OF FORMALDEHYDE CONCENTRATION

AGAINST TIME

Fig. XXa

30g. n-Al_2O_3 + 150ml. H_2O + 600mg. PFA

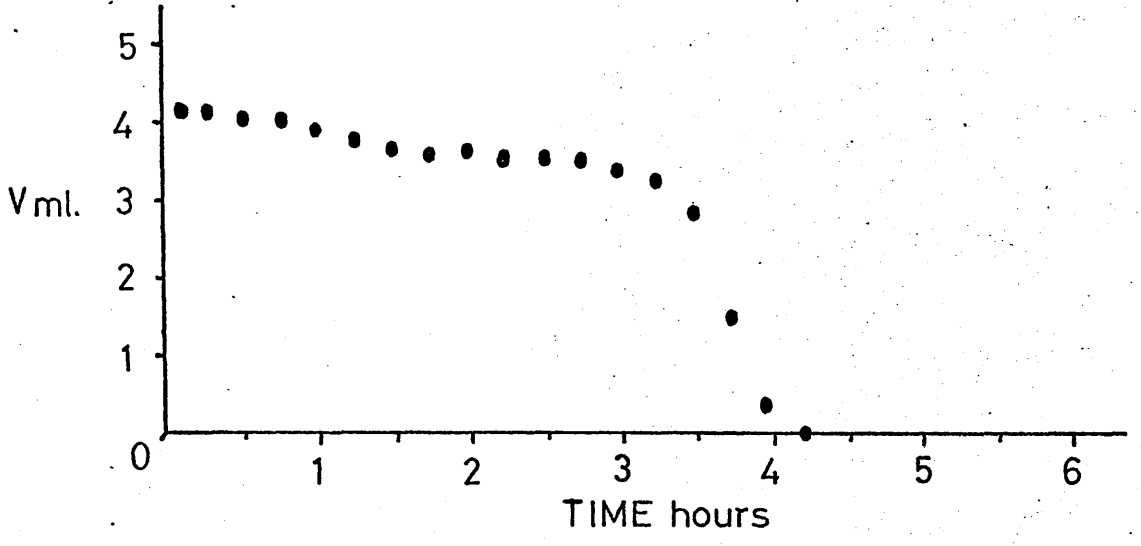
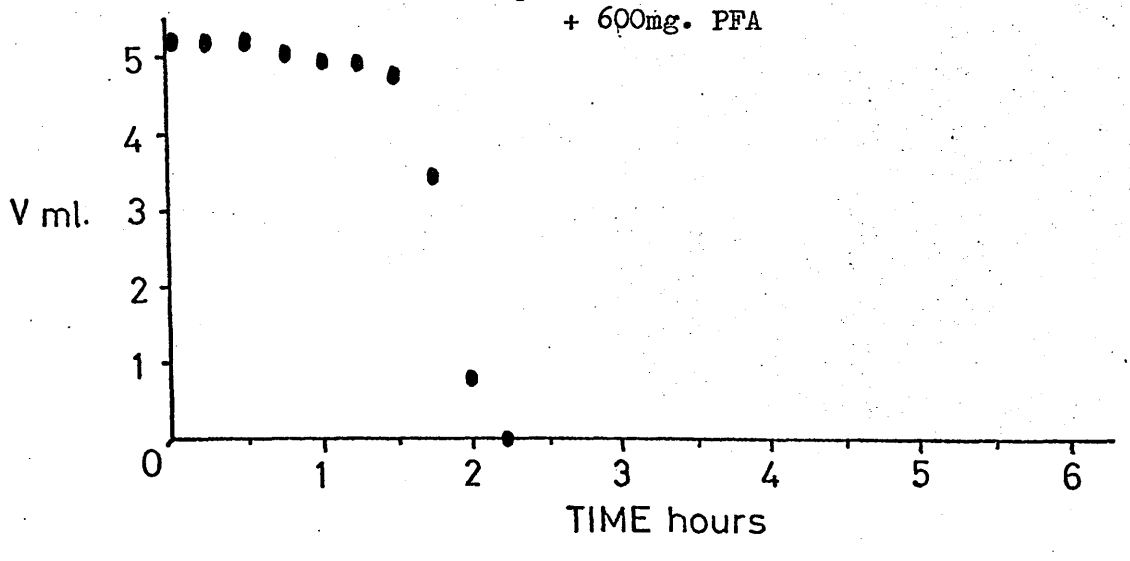


Fig. XX b

30g. n-Al_2O_3 + 150ml. H_2O reflux for $\rangle$ 24hrs.
+ 600mg. PFA



GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XXIa

a. 30g. a-Al_2O_3 + 150ml. 0.13M aq.PFA.

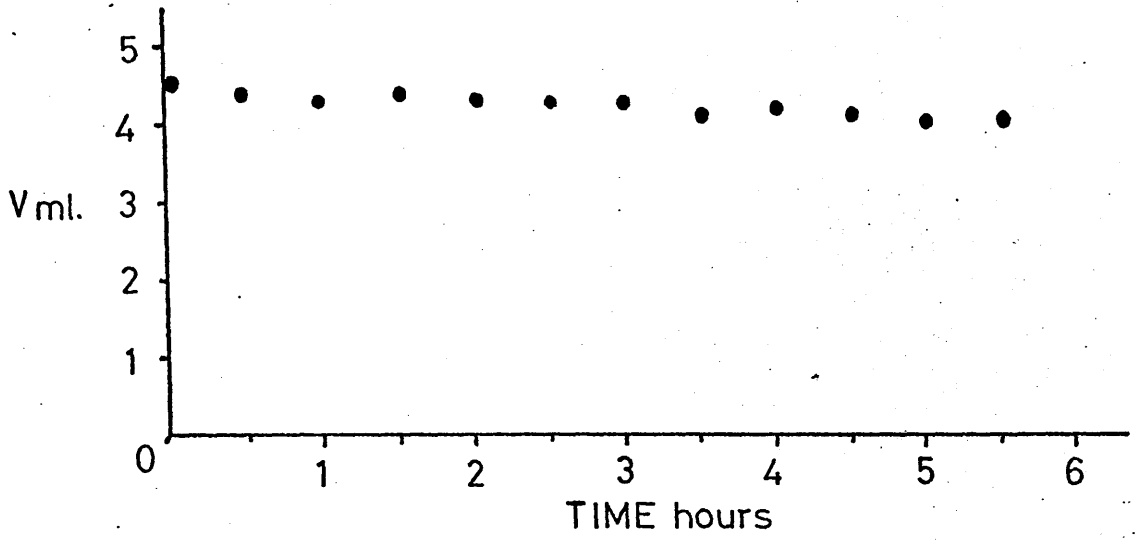


Fig. XXIb,c

b. 30g. b-Al_2O_3 + 150ml. 0.13M aq.PFA.

c. 30g. n-Al_2O_3 + 150ml. 0.13M aq.PFA.

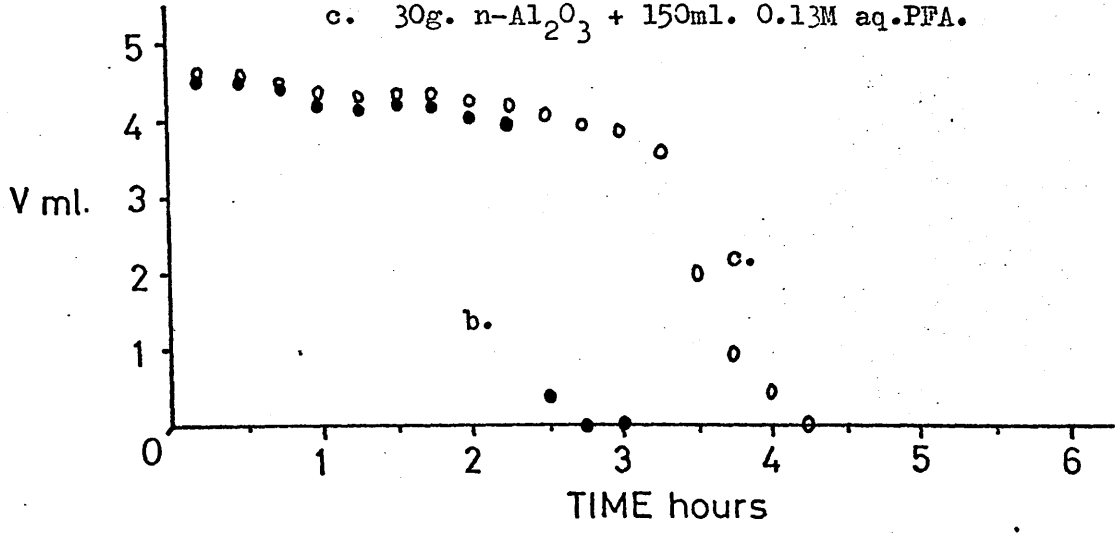


Table XXIIa

pH CHANGES ON REFLUXING ALUMINA IN WATER

ALUMINA TYPE	pH_1	pH_2	pH_3
acidic	4.2_2	4.3_0	4.3_0
neutral	8.0_0	8.7_0	8.8_5
basic	9.4_0	10.6_4	10.7_5

pH_1 after shaking for 1 hr. at room temperature

pH_2 after refluxing for 5 hrs.

pH_3 after refluxing for $22\frac{1}{2}$ hrs.

6.0g. Al_2O_3 + 30ml. H_2O

Table XXIIb.

pH DETERMINATIONS ON DIFFERENT AMOUNTS OF NEUTRAL ALUMINA IN WATER

MASS OF n-Al_2O_3 in 15ml. H_2O	pH_1	pH_2	pH_3
12.0g.	7.6	7.6	8.5
9.0g.	7.5	7.5	8.4_5
6.0g.	7.3	7.3	8.4_5
3.0g.*	6.9	7.0	8.4_5
1.5g.	6.4_5	6.9	8.4_5
0.5g.	6.3	6.7	8.2

* This corresponds to the scale of the standard reaction reduced by ten-fold

In the last experiment, 0.26M aqueous paraformaldehyde solution was added to the alumina and water to give a 0.13M solution. (Loss of water during the hydrating of the alumina in fact caused this concentration to be exceeded slightly.) In case any difference in lag time was reduced by the depolymerisation of the 0.26M polymers an identical type of formaldehyde was added to both neutral alumina freshly added to boiling water and the the same type of alumina that had been heated with water for at least 24 hours (Fig.XXa,b). Again this difference was shown. Comparison of Figs. XIXf and XXb, shows that depolymerisation is not a significant rate.

The reduction of the lag time by well-hydrated alumina might mean that pH is the controlling factor. The formaldehyde consumptions with acid, neutral and basic alumina were compared (Fig.XXIa-c). No formose formation was found with acid alumina: basic alumina was a more efficient catalyst than neutral. This is in accordance with expectation.

The pHs exerted by the three types of alumina were measured over a period of time (Table XXIIa). While the pH of acid alumina scarcely changed, it rose for the other kinds. Similarly pH increases were noted on leaving various amounts of neutral alumina in contact with water at room temperature. (Table XXIIb). We can see that pH rises as the amount of alumina is increased, and that after a considerable time has elapsed, that they approach a constant value. This may explain why a minimum lag time has been observed.

In experiment II, where the pH during the course of the formose reaction was monitored, the pH did not rise as much, because of the Cannizzaro reaction of formaldehyde which helped to keep it at low values. (More evidence for this will be given in the next Chapter).

GRAPHS OF FORMALDEHYDE CONCENTRATION AGAINST TIME

Fig. XXIIIa

100g. n-Al_2O_3 + 500ml. H_2O – reflux 10days
pH 8.7
Top 150ml of settled solution + 600mg. PFA

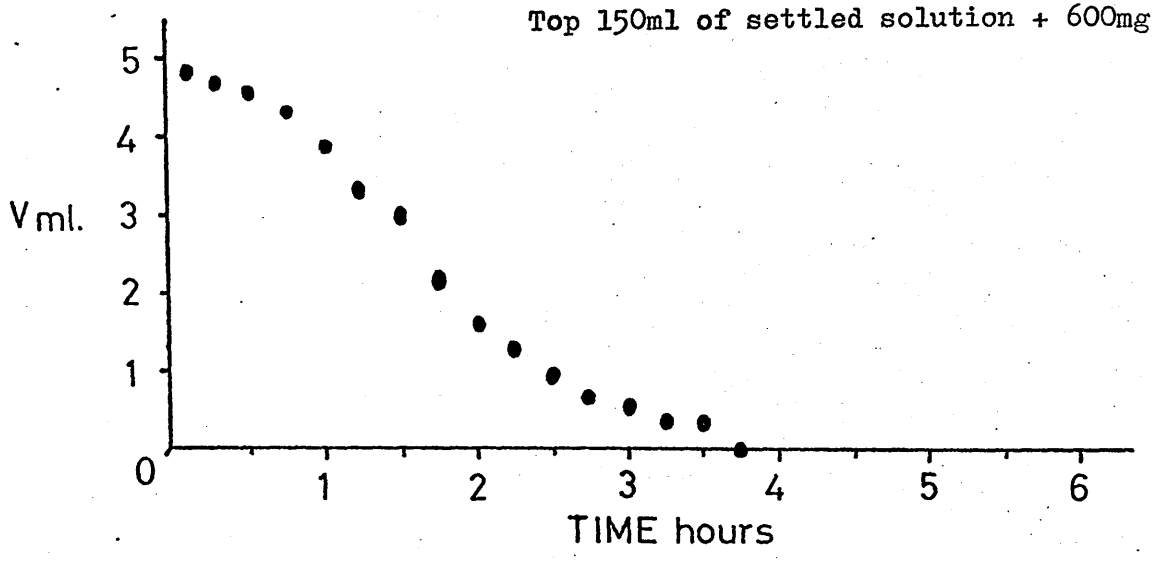
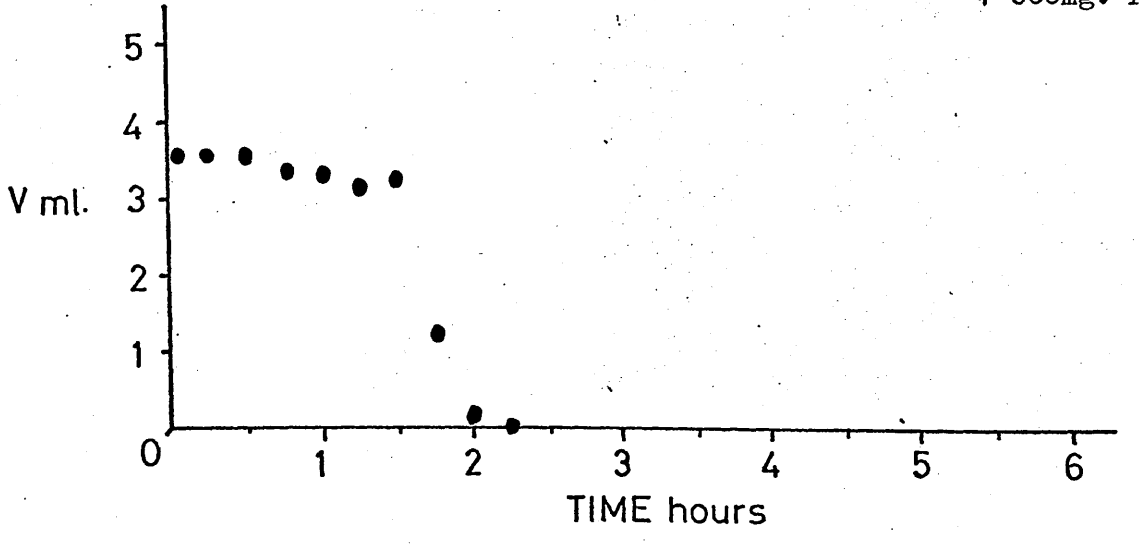


Fig. XXIII b

As above. Second 150mls of settled solution + 600mg. PFA



As the alumina hydrates in water, the solution becomes opaque. A greater surface area is presented by this alumina, therefore the total amount of alumina present in the flask should be able to be reduced without altering the rate of the formose reaction unduly. Experimental difficulties in the hydration of a small amount of alumina were encountered, because of the very slow rate at which the process occurs. A successful attempt to reduce the total amount of alumina was made in the following way.

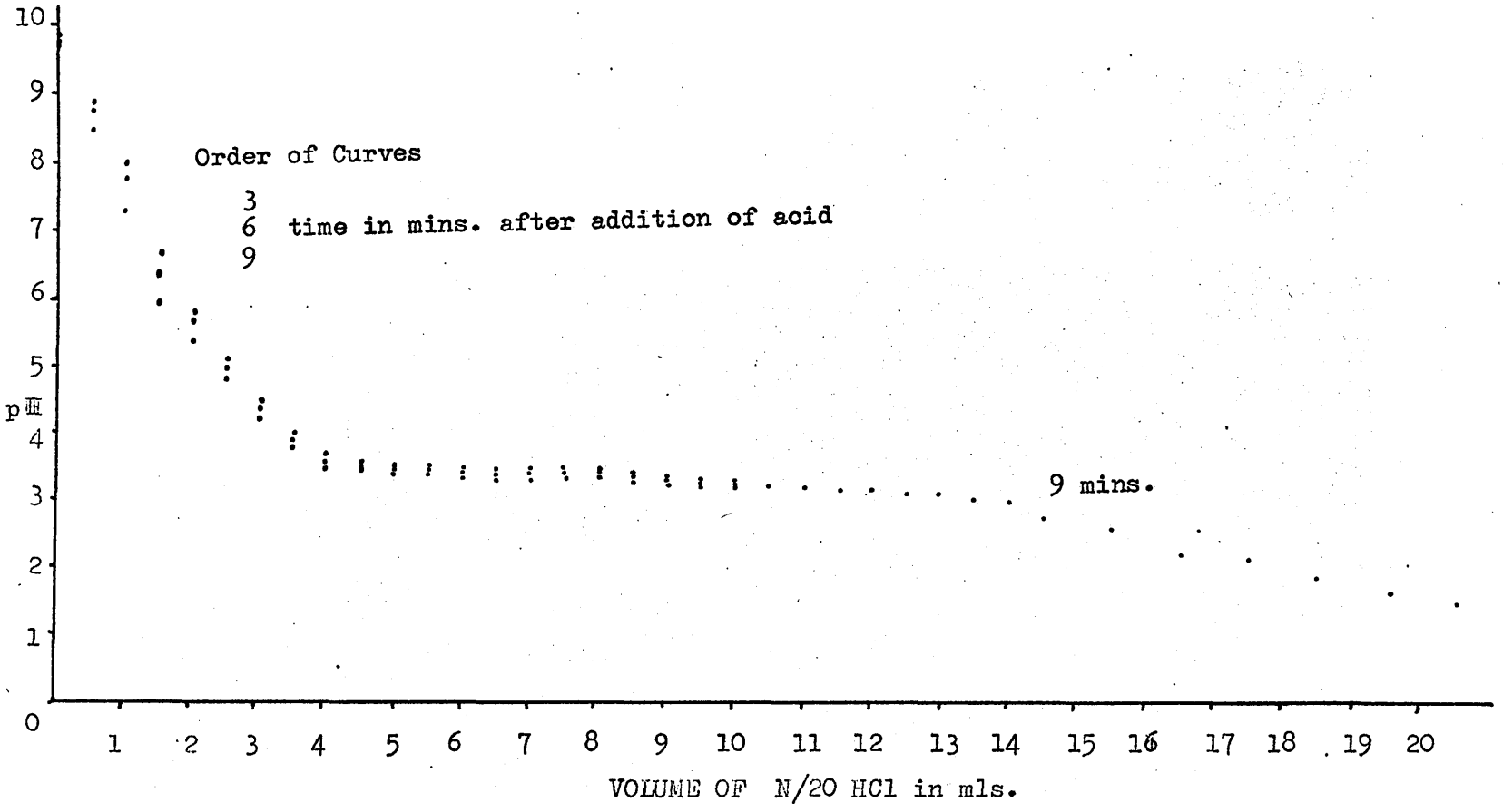
A large amount of alumina was heated with water to 100°, and maintained at that temperature for 10 days. At the end of this time the solution was opaque, from the aluminium hydroxide that had been formed. This mixture was then transferred to a measuring cy linder and allowed to settle. The solution was still milky; the top 150mls. and the next 150mls. were poured into separate flasks. After heating to reflux temperature, formaldehyde was added to each and its content followed (Fig.XXIIIa,b). In both cases, no formaldehyde remained after four hours. The pH for both solutions was initially the same, 8.7. Less than 1 g. alumina was present in both cases. Clearly with this reaction, the difference between homogenous and heterogeneous is being obscured.

We saw that acidic alumina did not catalyse the formose reaction, but that neutral and basic alumina did (Fig.XXIa,b,c). We may ask at what pH the change over between "formose" and "no" formose occurs, and whether it is sudden.

The experiment that should be attempted would need alumina containing solutions at different pHs, so that changes in the uptake of formaldehyde with pH could be measured.

Fig. XXIV

GRAPH OF CHANGES IN pH WITH TIME ON TITRATING BASIC ALUMINA WITH HYDROCHLORIC ACID



The extreme slowness with which alumina responds to changes in pH has been demonstrated by the titration of basic alumina with acid. Readings of the pH vary with time (Fig.XXIV). The pH changes on heating different kinds of alumina in water have already been mentioned (Table XXIIa). So, to alter the acid and base content of alumina in order to obtain a range of reliable intial pHs for such an experiment, is difficult and time-consuming, although in theory possible.

A series of experiments in homogenous sodium hydroxide solution was undertaken to find whether the change between "formose" and "no formose" would be sudden in that system. This and other similar experiments are described in Chapter 3.

Discussion

The Choice and Measurement of the Reaction Variable.

A variety of methods have been employed to follow the kinetics of the formose reaction, as we saw in Chapter I. The formaldehyde concentration is one of the most useful parameters, and is the method chosen for the examination of the formose reaction over alumina.

Aldoses and ketoses are made in the formose reaction, so a selective method of formaldehyde determination has to be used. Weiss has recently shown that the sodium sulphite and chromotropic acid methods of formaldehyde determination are suitable for use in this system.[133]

Of the two methods the former was found to be the more convenient and was used routinely. Methanol and formic acid, glucose, arabinose and furfural[216] do not interfere significantly with this method, although acetaldehyde does. Acetaldehyde has been added in one experiment (XIIp), but in small quantity.

The pH of the formaldehyde-containing solution must be approximately neutral, if the sodium sulphite method is to give reliable results. The presence of acid or alkali in this solution will affect the titration of liberated sodium hydroxide with hydrochloric acid. Neutral aluminium oxide exerts a pH of approximately 7½ in aqueous slurry and it would not be expected that significant interference would be found. Acid is produced during the course of the reaction, however, so the pH was measured during the course of the first kinetic experiment (II). The change was slight. (The minimum volume of solution that could be used for pH determinations was ca.5ml., consequently the scale of the experiment had to be suitably large. As this required a large amount of alumina (100g.) stirring was used to agitate the solid and so minimise any undesirable diffusional

effects. The scale of the reaction was reduced to the 30g. level in subsequent experiments, and pH measurements were not made. Here thermal energy was sufficient to agitate the alumina, so mechanical stirring was dispensed with.) Alumina has been titrated with sodium hydroxide (XXIV): there is no buffering region around pH 7.

Experimental Error in the Kinetic Curves.

A 2.00 ml. graduated pipette was used to remove the aliquots of solution for formaldehyde determination. As there is inevitably some alumina in suspension, this adds a slight error to the readings. Whenever alumina entered the pipette, the solution was allowed to settle for a few seconds before the alumina sediment was removed. The amount of alumina in suspension increased as the reaction progressed, because of the greater extent of alumina hydration.

A second difficulty arose when the alumina had hydrated to a considerable extent - the solution was slightly opaque. This whiteness, severe when the alumina and water had been boiled together for a time before the addition of formaldehyde, hindered the determination of the end point of the titration (the thymolphthalein indicator changes from blue to colourless as the acid is added).

In most cases the error on the estimation of alkali liberated by the reaction of formaldehyde with sodium sulphite is $\pm$ 0.1ml.

The Method of Starting the Reactions.

Heat for the flasks was supplied by a sandbath (Gallenkamp). The time taken to heat the formaldehyde solution from room temperature to 100° was usually at least a quarter of an hour. (On some occasions this was reduced by increasing the temperature setting of the sandbath e.g. XV and XVI, second parts. This was not done always

because of the danger of "bumping" - from experience it can be severe!) This time is significant, especially where accelerators are reducing the induction period. We therefore have a problem, how to assign a time to the start of the reaction.

Much less time is taken to add the alumina to the boiling formaldehyde solution, and this is the procedure that was adopted. Alumina was slowly added down the condenser. On its reaching the boiling liquid a vigorous ebullition occurred because nuclei were provided where boiling could start with less energy, and because the anhydrous alumina is being exothermically hydrated. There was considerable turbulence in the descending alumina even though the time for the addition was 20 - 30 seconds. A small amount of alumina adhered to the sides of the condenser. Accelerators were added immediately before, with or immediately after the alumina.

DETERMINATION OF FORMALDEHYDE BY THE SODIUM SULPHITE METHOD

Thymolphthalein Indicator Solution.

Thymolphthalein (BDH) was added to ethanol to make a 1% solution. At low pH the indicator is colourless, changing to blue at a higher value. The range of the indicator is 9.3 - 10.5.

Hydrochloric Acid Solution for Volumetric Analysis.

Standardised hydrochloric acid solution (N/20) was prepared by diluting the contents of the appropriate vial of Concentrated Volumetric Solution (BDH). (The N/10 vial was diluted to 1 litre instead of the recommended 500ml. This gave a solution of half strength, which was more convenient to use.)

Method.

12.6g anhydrous sodium sulphite were dissolved in 1 litre of solution (distilled water). The solution is M/10 in sodium sulphite. A 10ml. portion was transferred to a 50ml. Erlenmeyer flask with three drops of thymolphthalein solution. The solution turned blue, because of sodium hydroxide impurity in the sulphite. Hydrochloric acid was added until the blue vanished (approximately three drops of N/20 acid were required). The solution was then ready to receive the formaldehyde.

2.0ml. formaldehyde solution were added to the flask. The blue colour reappears because of the liberation of sodium hydroxide.

$$Na_2SO_3 + CH_2(OH)_2 = CH_2(OH).SO_3.Na + NaOH$$

The sodium hydroxide is estimated by hydrochloric acid titration. The volume of acid is proportional to the amount of formaldehyde. In the graphs the volume of acid is given by "V ml."

pH MEASUREMENT

A Pye model 78 pH - Meter was used, fitted with an Ingold E07 - 401 glass electrode. Buffer Solution Tablets (BDH) gave solutions of known pH, with which the meter was calibrated.

Materials.

Reagents for the Formose Reaction.

i Aluminium oxide.

The aluminium oxide was made by M.Woelm, Eschwege, Germany, and was of chromatography grade. Three types were used: "neutral" (n-), "acid" (a-) and "basic" (b-), all of activity grade I. Part specifications of the aluminas are given opposite. The abbreviations "a-Al_2O_3", "n-Al_2O_3" and "b-Al_2O_3" are used for acid, neutral and basic aluminium oxide, respectively.

Aluminium oxide (neutral) of other activity grades was prepared by the addition of distilled water to grade I material. The quantities are given in the opposite table. After addition of the water the alumina was shaken mechanically for 30 minutes and then left for a further 24 before use.

ii Formaldehyde.

Paraformaldehyde was supplied by British Drug Houses, Ltd., Poole (BDH).

$0.1\overline{3}$M aqueous formaldehyde solution was prepared by dissolving 14.0g. paraformaldehyde in 3.50 litres of distilled water under reflux. The solution was boiled for at least 24 hours after the disappearance of the solid. $0.5\overline{3}$M, $0.2\overline{6}$M and $0.1\overline{6}$M formaldehyde solutions were prepared in a similar manner, but on a reduced scale.

The solution obtained by dissolution of paraformaldehyde in water will be called "aqueous paraformaldehyde solution" (aq. PFA.) in order to distinguish it from formaldehyde solution prepared by dilution of "Formaldehyde Solution" ("AnalaR", BDH), which contains 11% methanol stabiliser. Apart from the alcohol there should be no significant difference between the composition of the solutions. Formaldehyde solution from the latter source is used in experiment X. The composition of both solutions is mainly methylene glycol (see text of this Chapter).

ALUMINIUM OXIDE - PARTIAL SPECIFICATION

(From Technical Data supplied by M.Woelm)

TYPE	APPROXIMATE pH	PARTICLE SIZE
ACID	4	50 - 200μm
NEUTRAL	7.5	50 - 200μm
BASIC	10	50 - 200μm

ACTIVITY GRADES OF ALUMINA (NEUTRAL)

ACTIVITY GRADE	% DISTILLED WATER TO BE ADDED TO n-Al_2O_3	MASS OF ALUMINA OF NEW GRADE CONTAINING SAME AMOUNT OF ALUMINIUM AS 30g. GRADE I
II	3	31g.
III	6	32g.
IV	10	$33\frac{1}{3}$g.
V	15	35g.
VI	21	37g.

The Experiments.

Numbers assigned to figures, graphs and tables correspond with the number of the experiments.

I a. 6.0g. n-Al_2O_3 + 30ml. 0.13M aqueous paraformaldehyde solution (aq. PFA.)

30ml. 0.13M aqueous paraformaldehyde solution were refluxed for five hours in the presence of 6.0g. neutral aluminium oxide under an atmosphere of nitrogen. After cooling the alumina was removed by filtration, and an aliquot of the solution taken for the qualitative estimation of formaldehyde (Chapter V). No formaldehyde remained. An aniline oxalate spot test on the concentrated solution confirmed this finding (Chapter V). The concentrated solution was chromatographed on paper, and a variety of sprays, specific for sugar usually, were used to locate the products. A list of the R_{glu} values of the spots obtained by use of the aniline oxalate spray is given in the text. (Details of the sprays and chromatographic procedures are given in Chapter V.)

At the start of the reaction the alumina is white, and this colour presists until after about three hours a yellow colour starts to appear. The odour of caramel is also noticeable about this time.

I b. 30ml. 0.13M aqueous paraformaldehyde solution were adjusted to pH 7.4 (corresponding to that exerted by a slurry of neutral alumina) with sodium hydroxide and hydrochloric acid. After five hours of refluxing the procedure in Ia was adopted.

Formaldehyde remained at the end of the reaction and no sugars were found.

I c. 6.0g. n-Al_2O_3 were extracted with 30ml distilled water

at 100° for five hours. The procedure is as in Ia. Neither sugar nor formaldehyde was detected.

Gabel and Ponnamperuma used 10g. alumina and 50ml. 0.33M formaldehyde solution.

II 100g. n-Al_2O_3 + 500ml H_2O + 2.00g. paraformaldehyde refluxed under nitrogen with stirring.

500ml distilled water were placed in a 1 litre 3-necked flask equipped with a paddle stirrer and reflux condenser. Nitrogen was passed through the system for an hour prior to the next stage. The paraformaldehyde was added, allowed to dissolve and the resulting solution heated for two hours. A 5.0 ml. aliquot was removed and cooled. The pH of this was then measured at room temperature and a 4.0_0 ml portion then removed for formaldehyde determination by the sodium sulphite method.

III 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. under nitrogen , without stirring.

The alumina and formaldehyde are in the same ratio, and the formaldehyde at the same concentration as in experiments Ia, and II but the scale of the reaction has been changed.

2.0_0 ml. aliquots of the solution were removed at intervals for formaldehyde determination by the sodium sulphite method.

IV 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA in air.

This reaction is identical to III except that no nitrogen was present.

The conditions used in this experiment are often repeated. We shall regard these conditions as being "standard".

A slight variation in the length of the lag time has been encountered: where a comparison must be made with a standard e.g. when we are examining the effect of compounds that may be

accelerators, a reference curve will be supplied. The reference curve will have been obtained using the same batch of alumina and will often have been obtained at the same time as the other curve. In other cases the reference curve will have been obtained with the same temperature setting of the sandbath. (It was belatedly found that the temperature of the sandbath could have a marked effect on the lag period. The higher the temperature, the shorter the induction time. It was not possible to heat all the flasks at exactly the same rate because, firstly, they were all in contact with the sandbath to exactly the same extent, and secondly and more seriously, some of the flasks were prone to bumping. This was unpredictable and when it occurred the heating rate had to be decreased.)

V 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 1mg. of the following:

a. D-glyceraldehyde
b. 1,3-dihydroxyacetone
c. D-ribose
d. D-glucose
e. D-fructose
f. sucrose
g. soluble starch.
h. 4mg formose (added as 1 ml. formose from the standard reaction after 5 hours, and calculated on the basis of 100% conversion of formaldehyde to sugar.)

h' 150ml 0.13M aq. PFA. at pH 7.4 (NaOH-HCl) + 4mg formose (above)

a' - g' The conditions are identical to a - g except that the reaction is carried out in the absence of alumina, in formaldehyde solution at pH 7.4 initially. The formaldehyde concentration was not followed in these cases, but estimations of the aldehyde content at the end of the reactions showed that virtually no formaldehyde was consumed. The solutions were also clear.

VI 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA +

a. 3.6g. D-glucose

b. 18g. D-glucose.

a' 30g. n-Al_2O_3 + 3.6g. D-glucose + 150ml. H_2O
No formaldehyde was detected by the sodium sulphite method after 5 hours of refluxing. The presence of such a large amount of glucose did not interfere with the method of formaldehyde determination.

c. 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. Refluxed for 24 hours before the addition of 600mg. paraformaldehyde. The formaldehyde concentration was then followed.

d. The product from c. was refluxed for a further 15 hours and 600mg paraformaldehyde were added. The formaldehyde concentration was again followed.

VII 30g. n-Al_2O_3 + 150ml.0.13M aq. PFA +

a. 0.01mg D-fructose (added in a 0.1mg.ml.$^{-1}$ solution)

b. 0.001mg D-fructose (" " " " ").

VIII 30g. n-Al_2O_3 + 150ml.. 0.13M aq. PFA. +

a. 1.0mg glycolaldehyde (added in a 1 mg.ml.$^{-1}$ solution)

b. 0.01mg "

c. 0.001mg "

IX 30g. n-Al_2O_3 + 30ml. distilled H_2O with

a. 1.0mg glycolaldehyde (added in a 1 mg. ml.$^{-1}$ solution)

b. 0.1mg "

c. 0.01mg "

X 30g. n-Al_2O_3 + 148.5ml.H_2O + 1.5ml 13.3M Formaldehyde Solution

30g. n-Al_2O_3 were added to 148.5ml refluxing distilled water, followed by 1.50ml. 13.3M Formaldehyde Solution ("AnalaR",BDH). This gave a solution 0.13M in formaldehyde. 0.5ml. samples were withdrawn at intervals and filtered through cotton wool to remove most of the alumina in suspension. After dilution by tenfold a 0.005ml sample was withdrawn, and added to 10.0ml chromotropic acid reagent. After heating all the chromotropic acid samples together for 15 minutes at 100°, the ultra-violet absorbances at 570 - 580nm. were measured.

XI 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. + 130mg KCN.

10% molar relative to formaldehyde of potassium cyanide ("AnalaR", BDH) was added.

XII 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA. +

a. 42.5mg. benzoin
b. 42.5mg. benzoin + 13mg. KCN (added together .)
c. 21mg. benzaldehyde
d. 21mg. benzaldehyde + 13mg. KCN (added separately)
e. 15mg. acetol (made by hydrolysing 18mg 1-chloroacetone in 1 ml. H_2O for 30mins prior to reaction)
f. 38mg. 3-hydroxy-3-methylbutan-2-one
g. 35mg. 4-hydroxybutan-2-one
h. 23mg. diacetone alcohol
i. 20mg. acetylacetone (redistilled)
j. 42mg. benzil
k. 17mg. diacetyl (redistilled)
l. 47mg. 2,3,4,6-tetra-O-methylglucose
m. 39mg. methyl-α-D-glucopyranoside
n. 78mg. penta-O-acetylglucose
o. 33mg. 2-deoxyglucose
p. 9mg. acetaldehyde (redistilled)
q. 34mg. L-ascorbic acid
r. 28mg. kojic acid

XII cont.

s. 29mg. 8-hydroxyquinoline

t. 22mg. catechol

All compounds were added in 1% relative to formaldehyde.

XIII Variation of amounts of basic alumina and formaldehyde.

The quantities are recorded in Table XIII. Each was added to 30ml. distilled water, before refluxing for 5 hours was started. Formaldehyde at the end was estimated qualitatively by the chromotropic acid method.

XIVa. 100mg. Al_2O_3* + 150ml. 0.13M aq. PFA

b. 1.0g. Al_2O_3* + 150ml. 0.13M aq. PFA

c. 100mg. Al_2O_3* + 29.9g. n-Al_2O_3 + 150ml. 0.13M aq. PPFA

d. 1.0g. Al_2O_3* + 29.0g. n-Al_2O_3 + 150ml. 0.13M aq. PFA

Al_2O_3* was obtained by washing alumina from the end of a standard reaction with distilled water, and drying it at the water pump prior to the start of these reactions.

XV a 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA.

The formaldehyde content was followed until the lag period had ended. The flask was then rapidly chilled in ice, and the phases separated; these will be termed the "old" reagents. The "old" alumina was well washed with water.

b 70ml. "old" solution + 14g. n-Al_2O_3

c 30g. "old" Al_2O_3 + 150ml 0.13M aq. PFA

The time from chilling to reheating was 75 mins. : 15 mins were then required for the flasks to heat to reflux temperature.

The "fresh" reagents were added in order to preserve the original ratios of reagents.

XVI a,b,c as above.

d. 40ml "old" solution only.

XVII a 30g. n-Al_2O_3 + 150ml.0.53M aq. PFA
b 30g. n-Al_2O_3 + 150ml.0.27M aq. PFA
c 30g. n-Al_2O_3 + 150ml.0.13M aq. PFA
d 30g. n-Al_2O_3 + 600ml.0.13M aq. PFA

In a. 15mls. sodium sulphite reagent were used.

XVIIIa. 60g. n-Al_2O_3 + 150ml.0.13M aq. PFA
b. 30g. n-Al_2O_3 + 150ml.0.13M aq. PFA
c. 15g. n-Al_2O_3 + 150ml.0.13M aq. PFA
d. 5g. n-Al_2O_3 + 150ml.0.13M aq. PFA
e. 1g. n-Al_2O_3 + 150ml·0.13M aq. PFA

XIX a 31g. n-Al_2O_3(grade II) + 150ml.0.13M aq. PFA
b 32g. n-Al_2O_3(grade III)+ 150ml.0.13M aq. PFA
c 33$\frac{1}{3}$g. n-Al_2O_3(grade IV) + 150ml.0.13M aq. PFA
d 35g. n-Al_2O_3(grade V) + 150ml.0.13M aq. PFA
e 37g. n-Al_2O_3(grade VI) + 150ml.0.13M aq. PFA
f 30g. n-Al_2O_3 + 75ml H_2O: reflux for 5 days: add 75ml 0.26_6M aq. PFA (giving 0.13M CH_2O)

In f. the solution was very milky, consequently end-points were more difficult to determine than usual.

XX a 30g. n-Al_2O_3 + 150ml. H_2O + 600mg. PFA
b 30g. n-Al_2O_3 + 150ml. H_2O: reflux for 24hrs: add 600mg PFA (giving 0.13M CH_2O)

XXI a 30g. a-Al_2O_3 + 150ml.0.13M aq.PFA
b. 30g. b-Al_2O_3 + 150ml.0.13M aq.PFA
c. 30g. n-Al_2O_3 + 150ml.0.13M aq.PFA

XXII pH Determinations on alumina.

a. 6.0g. a-, n-, b-Al_2O_3 + 30ml. H_2O
pHs determined at intervals (see Table XXIIa)

b. Various masses of n-Al_2O_3 were added to 15ml. H_2O
pHs determined at intervals (see Table XXIIb)

XXIII 100g. n-Al_2O_3 + 500ml. H_2O: refluxed for 10 days.

At the end of this time the pH of the solution was 8.7. The mixture was transferred to a 500ml. measuring cylinder and allowed to settle for 10 mins., the total volume being 410ml.. (Some water was lost through evaporation during the prolonged reflux period.)

a. The top 150ml. of the settled solution were decanted into a 500ml. flask and 600mg. PFA were added to the solution after it had been heated to 100°.

b. As a., except that the second portion of 150ml was used.

The formaldehyde molarity in both of these solutions was 0.13.

Titration end-point determinations were difficult due to the milkiness of the solutions.

XXIV Titration of basic alumina.

1.00g. b-Al_2O_3 + 10ml. distilled H_2O were stirred with a by a magnetic follower, and when the pH had settled a reading was taken.

N/20 HCl was added in 0.50ml. portions, and pH readings were atken every 3, 6 and 9 minutes after the addition of the acid. Successive aliqouts of acid were added every 10 minutes.

CHAPTER 3

THE DEPENDENCE OF THE FORMOSE REACTION ON pH

Introduction.

In the previous chapter we saw that while neutral and basic alumina were formose reaction catalysts, acidic alumina was not. It is reasonable to attribute this difference to the pH of the reaction media.

At what pH does the formose reaction start, and how sudden is the change from "no formose" to "formose"?

Experimental difficulties with the aluminium oxide system (arising from the slow attainment of dissolution equilibria) led to preliminary investigations being made in the sodium hydroxide system, which is homogeneous.

Discussion.

Sodium hydroxide has been used as a formose catalyst several times (Chapter 1, Table I). It is also a Cannizzaro reaction catalyst (Appendix IV), and is said to be more suited to the latter reaction than to the former.[93]

A number of solutions containing the same number of gram ions of sodium but at different pHs were prepared. The pHs ranged from 2 to over 12. Equal quantities of formaldehyde were added to each solution (0.13M in formaldehyde), and the solutions were refluxed for five hours on a sand-bath (I). Within an hour or so the solutions in a few of the flasks at highest pH had turned brown or yellow, the intensity of the colour being greatest in the flasks at the highest pHs. The times of appearance of the colours (to the eye) were in order of

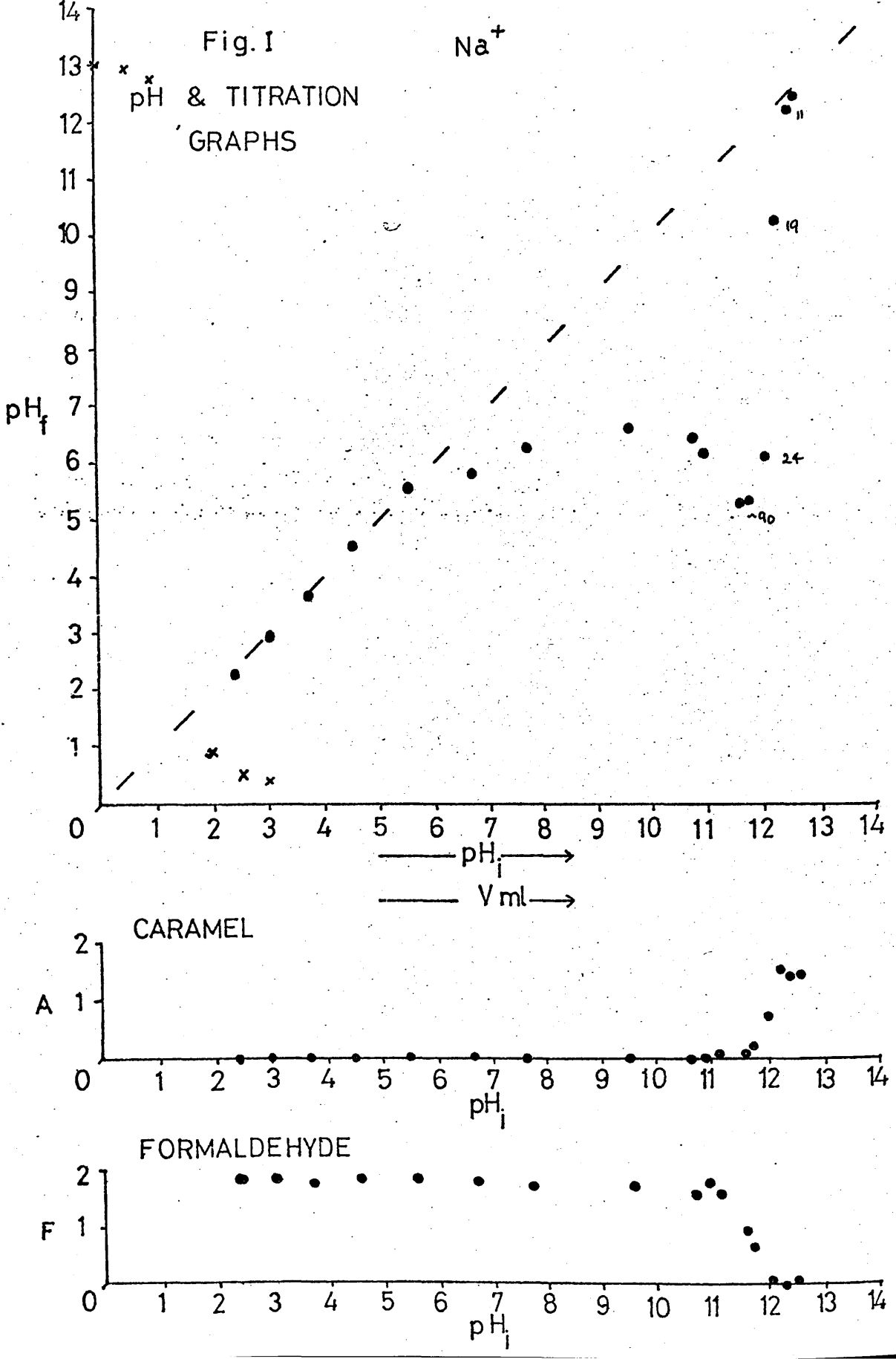
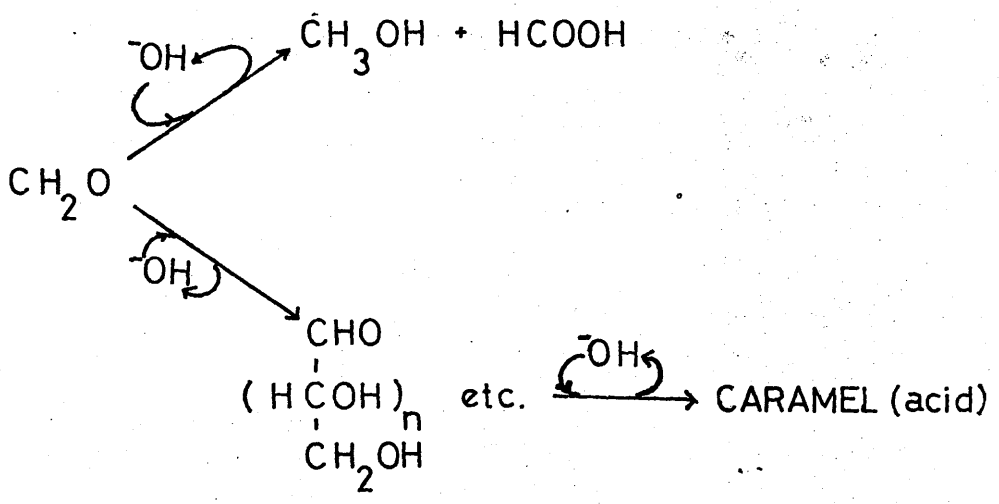


Fig. I
Na+
pH & TITRATION GRAPHS
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

TABLE A.

BASE - CATALYSED REACTIONS OF FORMALDEHYDE



descending pH. After five hours the flasks were removed from the sandbath, cooled and the final pH measured. Fig. I shows the pH changes that were found. The amount of formaldehyde (chromotropic acid method) and the absorption maxima for caramel (in the range 225 - 275 nm.) were also recorded. (The caramel absorption should be taken only to give an indication of when sugar had been formed, as the absorption value changes with pH. This is mentioned in the Experimental Discussion.)

Formaldehyde may undergo a number of base-catalysed reactions (Table A) and we may interpret Fig. I in terms of these.

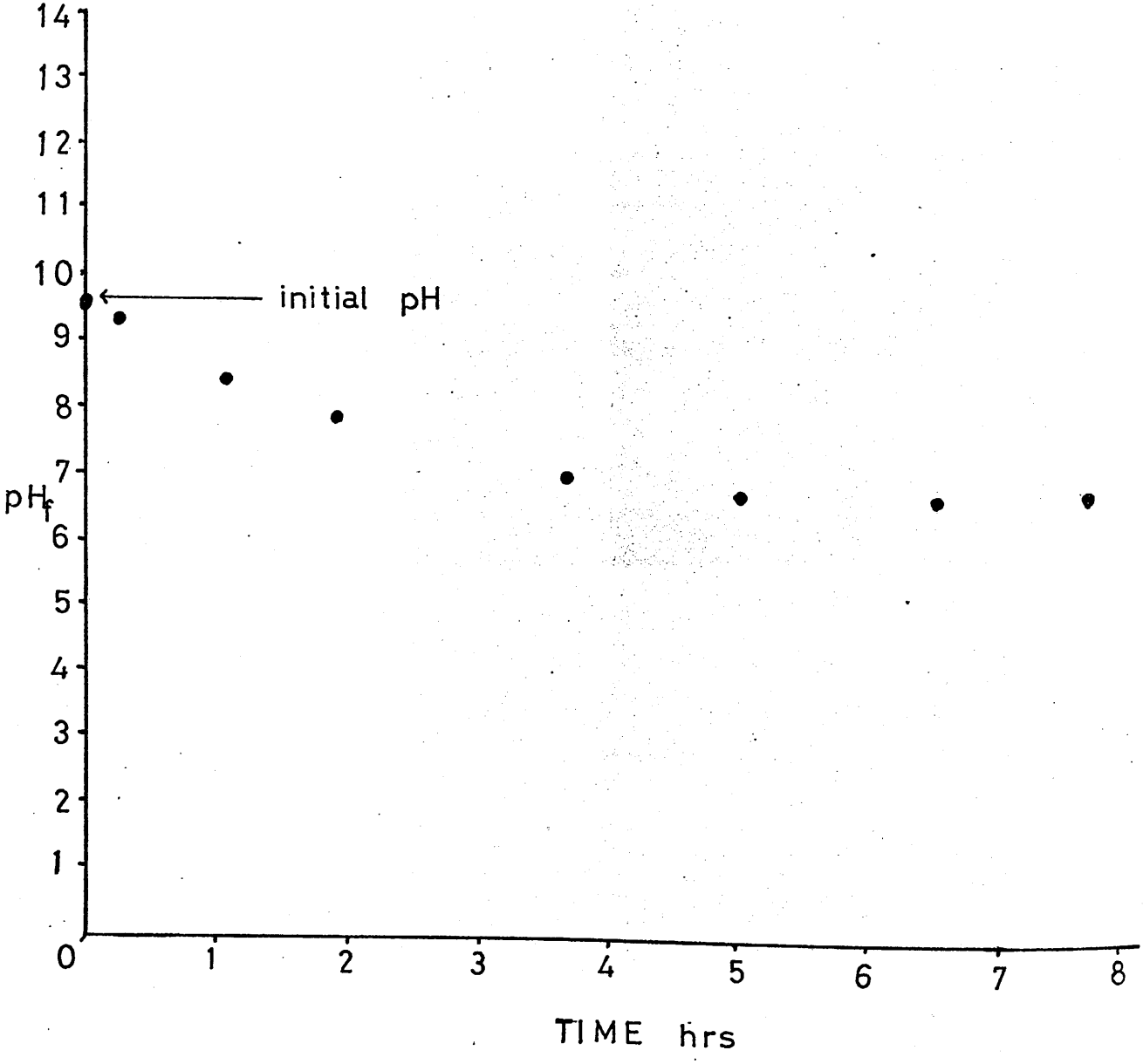
The initial pH does not significantly change at values less than 6: neither acid nor alkali has been produced. From initial pH values of 6 to 11.5 the final pH has an approximately constant value 6.2 $\pm$ 0.4, most of the formaldehyde remains and no sugar (or caramel) has been formed. The Cannizzaro reaction resulting in the production of formic acid, is the most probable explanation for this, the reaction stopping (or "switching off") at pH 6.2.

Above pH_i 11.5 the final pH shows less and less of a departure from the initial value: the formaldehyde concentration falls rapidly and the caramel absorption rises with the increase in pH. We may take the appearance of an absorption in the 225 -250nm. region as an indication of the presence of sugar. (Results from the heating of glucose solutions at a range of pH values will be mentioned later.) In the pH_i region above 11.5 the formose takes over from the Cannizzaro reaction until virtually no Cannizzaro reaction has occurred. We may say that the formose reaction has "switched on".

During the induction period for the reaction, the Cannizzaro reaction occurs at a greater rate than the formation of glycolaldehyde, but when the second stage of the formose reaction begins, the rate of this autocatalytic process supercedes the rate of the Cannizzaro reaction. We might expect that had the formose reaction not been a possible reaction of formaldehyde, that the final pH above pH_i 11.5 would have been approximately 6.2 until insufficient

GRAPH OF pH AGAINST TIME

Fig II Na^+ pH 9.5



formic acid was produced to lower the final pH to this value. This, of course, would depend on the amount of formaldehyde added initially.

The appearance of a minimum in the graph is slightly puzzling. Sugars would have been produced below pH 11.5, albeit in small amount (there is evidence for this in the tetramethylammonium hydroxide-catalysed reaction, later.) Aldoses and formaldehyde would undergo a cross-Cannizzaro reaction, resulting in the formation of formic acid, and perhaps give a different value for the "switching off". The effect of aldose replacing one formaldehyde molecule in Pfeil's Cannizzaro complex (Appendix IV), and causing a hydride transfer at a lower pH than when formaldehyde alone was present, has yet to be explained. Caramelisation may cause a different minimum.

A tacit assumption that has been made so far is that after five hours of refluxing all the systems have reached an equilibrium value, apart from caramelisation which will continue to lower the pH. The appearance of a maximum at pH_i 9.5 suggests that further refluxing might lower the value some more. A series of flasks containing sodium hydroxide solution at pH 9.5 and equal amounts of formaldehyde were heated under reflux, and at intervals, were removed in order to measure the change in pH. Fig.II shows the change in pH with time. The minimum value is attained in five hours and further heating does not lower the value.

Pfeil noted that a high formaldehyde-to-catalyst ratio favoured the Cannizzaro reaction.[78] If we increase the amount of formaldehyde added to the sodium hydroxide system we might expect to see the Cannizzaro reaction occurring in place of the formose reaction (this resembles, in some respects, the earlier hypothesis that we made, of what would happen if the the formose reaction did not exist).

Ten times the quantity of formaldehyde added in experiment I was added to a series of flasks containing sodium hydroxide and hydrochloric acid at various pHs. The graph of pH_i against pH_f

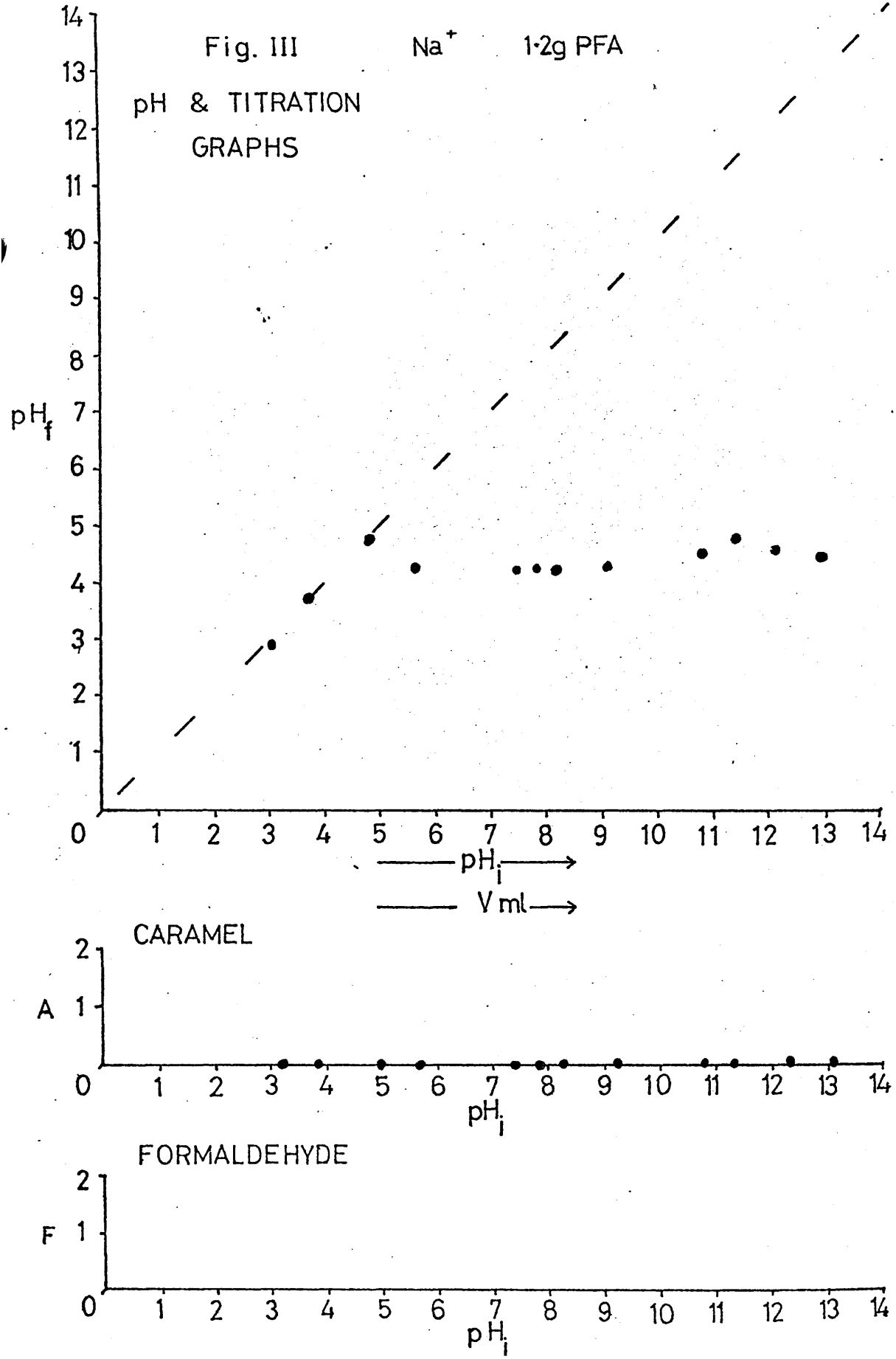


Fig. III
Na+
1·2g PFA
pH & TITRATION
GRAPHS
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

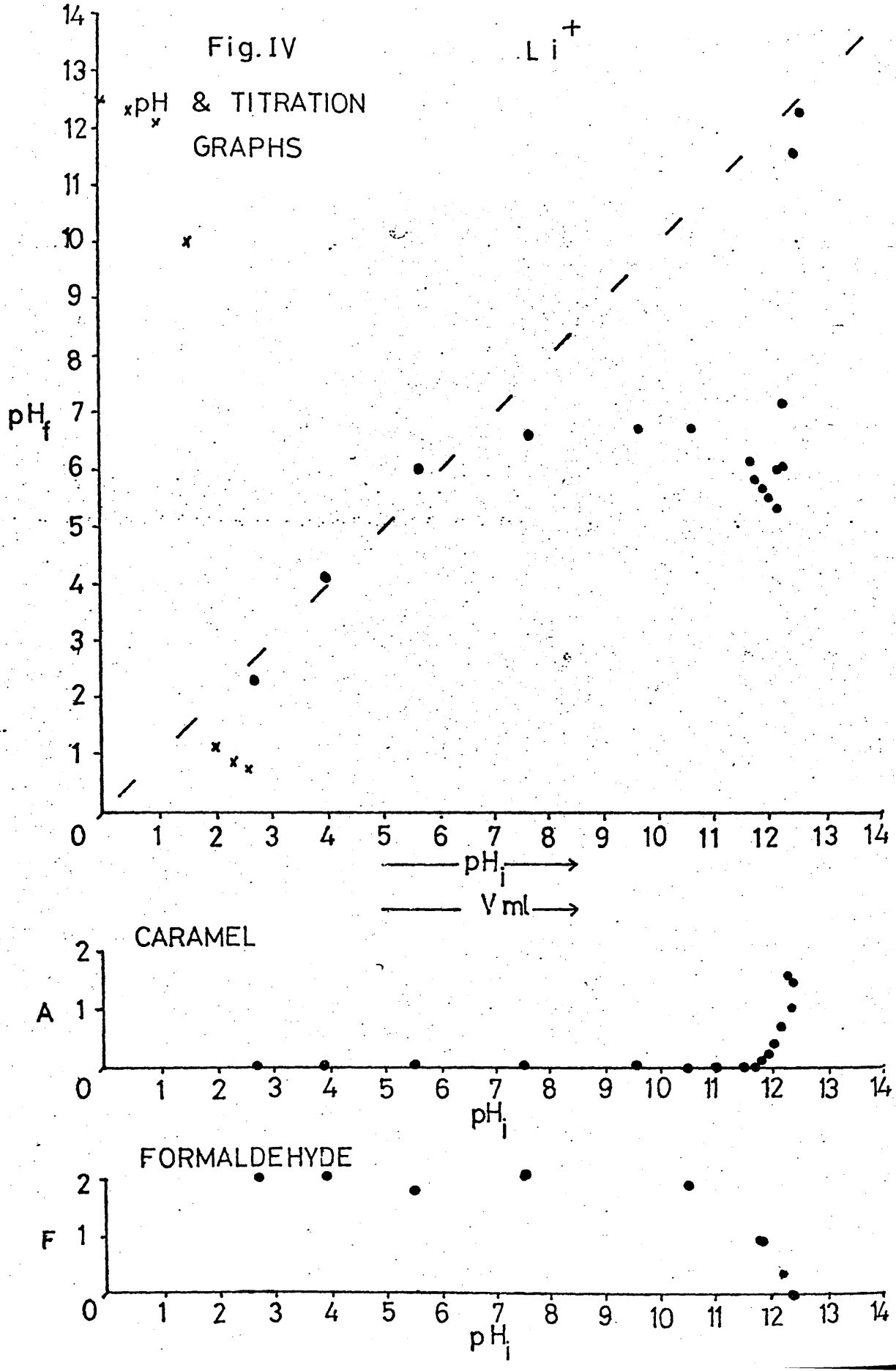


Fig. IV
Li+
pH & TITRATION GRAPHS
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

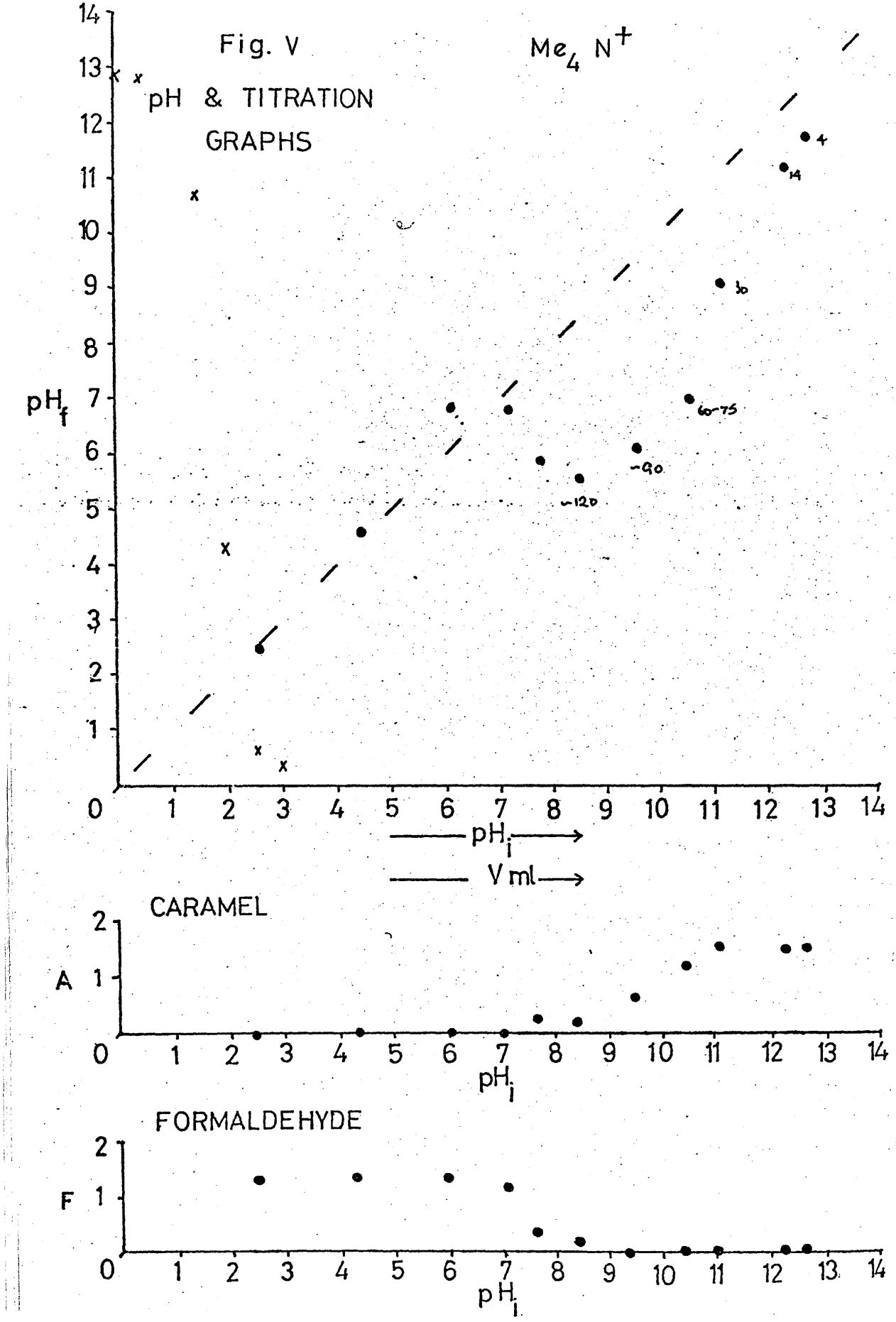


Fig. V
pH & TITRATION GRAPHS
$Me_4 N^+$
pH_f
pH_i
V ml
CARAMEL
A
pH_i
FORMALDEHYDE
F
pH_i

is given in Fig. III. The final pH was approximately 4.5, an in all cases the solutions were colourless, even up to pH_i 13. No sugar was formed. The reason for the new minimum is not clear.

Let us now see what other ions do to the curve. Lithium hydroxide-hydrochloric acid solutions were prepared, in a similar manner to the solutions for experiment I. Fig. IV shows the changes that have been found. The curve has a very similar shape to the one given by sodium ions, as expected. The Cannizzaro "switch off" value is almost the same, but the change to formose production is found at a slightly higher value.

The tetramethylammonium ion has the ability to give solutions of high pH, but without the possibility of co-ordination of any species to the cation. Like sodium and lithium, the tetramethylammonium ion gives homogeneous solutions in the pH range which we are studying.* The changes in pH caused by this ion are shown in Fig. V. We immediately notice that the curve has a different shape, and that sugar has been formed at a much lower pH - down to about pH 8. Why should this be so? By using the tetramethylammonium ion we have removed the possibility of co-ordination of formaldehyde to the cation of the solution. Any mechanism that requires co-ordination as a necessary part will not be feasible: conversely, any mechanism which does not need it will still happen. In this solution the hydroxide ion will be the main catalyst. That the formose reaction occurs suggests that co-ordination is not a necessary part of it. That it occurs at such a low pH suggests that the Cannizzaro reaction has been, at least, hindered. The implication from this is that the latter reaction requires co-ordination. The pH has fallen above pH_i 7. This may be due to caramelisation of the sugars that have been formed, or perhaps to the Cannizzaro reaction that is occurring at a much reduced rate because of the absence of co-ordination.

* Titration curves are denoted by "x".

If we apply these results to the sodium and lithium hydroxide systems, we can see that co-ordination must occur for the Cannizzaro reaction. Why are sugars not formed at such a low pH in these systems? Clearly, they must be formed, but in small amounts because of the greater rate of the Cannizzaro reaction, which lowers the pH to a level where the formose reaction cannot occur.

There are three reports in the literature about the ability of the tetramethylammonium ion to catalyse the formose reaction (see Chapter 1). The results we have so far obtained, suggest why one of these reports is negative. Either an insufficient pH has been given to the solution, or, more likely, too much formaldehyde has been added.

On Fig. V the times of appearance of a yellow colour have been recorded. It can be seen that the induction period increases as the pH is lowered. The rate of browning is highest in those solutions of the largest value of pH. Browning times will be entered on many of the following graphs: the units are minutes.

So far we have been examining the catalysis by monovalent cations in homogeneous basic solution. When we turn to other ions, such as magnesium, titanium and zinc, the solutions are found to be heterogenous over some pH ranges, and the possibility of bufferring effects caused by these polyvalent ions must be considered. For practical reasons (Experimental Discussion) sodium hydroxide was added to solutions of the (soluble) chlorides of the following ions. The participation of sodium ions in determining the shape of the curves should not be overlooked.

Titration curves of the chlorides of the metals with sodium hydroxide are placed beside the pH curves for easier comparison. When the solution becomes heterogeneous the $\bigcirc\!\!\rightarrow$ sign will be used, the heterogeneous solution lying to the side of the arrow.

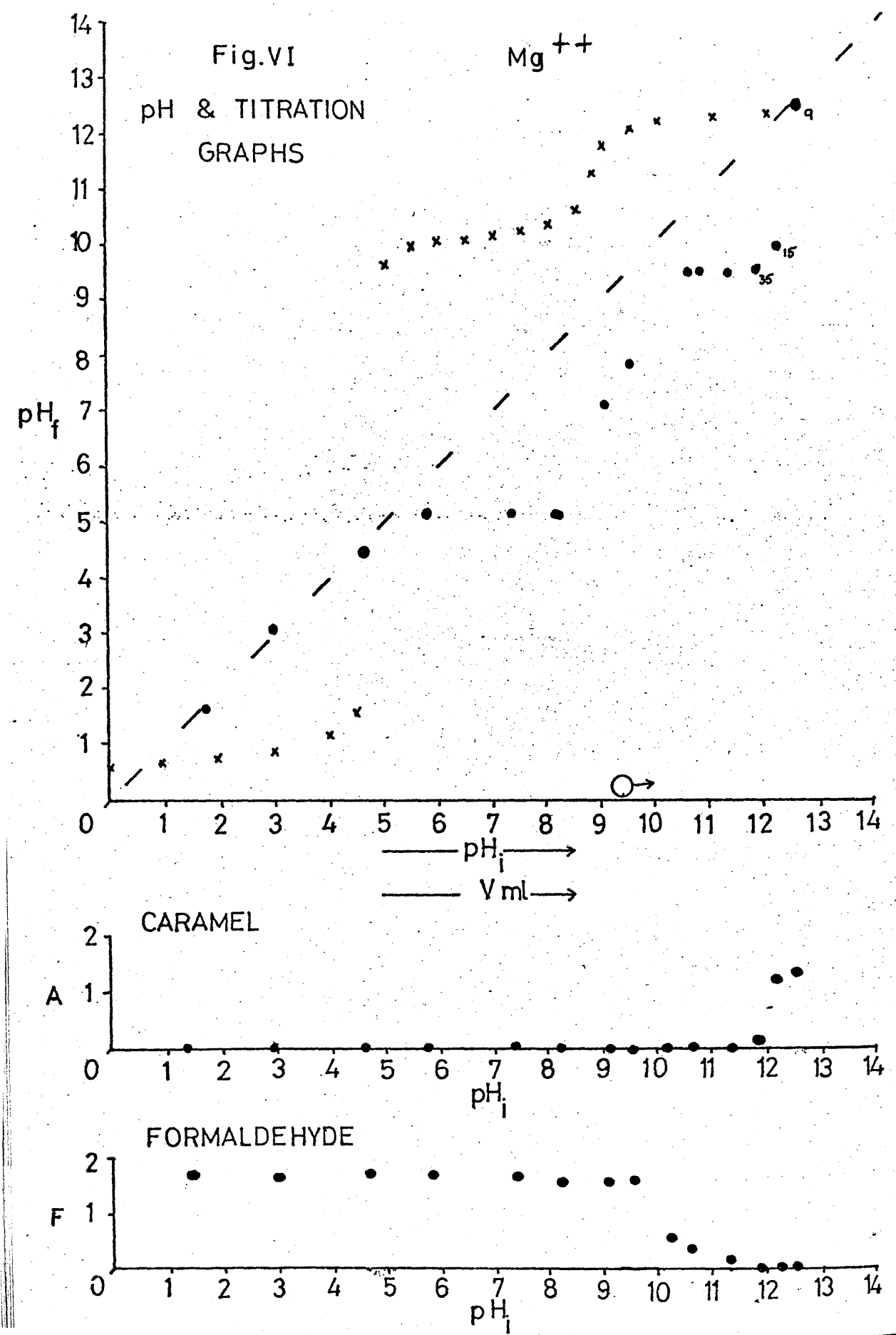


Fig.VI
pH & TITRATION
GRAPHS
Mg++
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

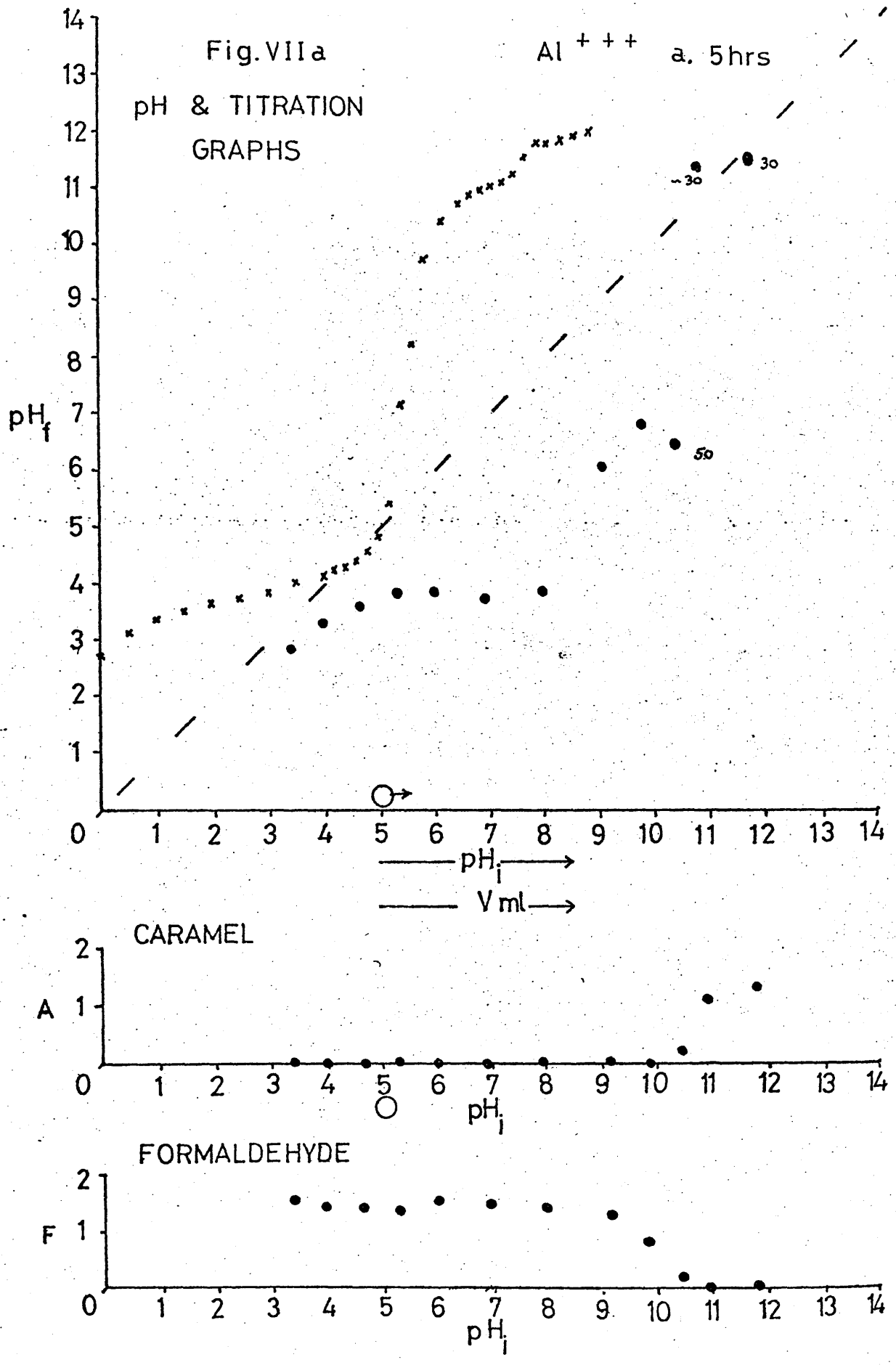


Fig. VII a
Al +++ a. 5 hrs
pH & TITRATION GRAPHS
30
30
50
pH_f
pH_i
V ml
CARAMEL
A
pH_i
FORMALDEHYDE
F
pH_i

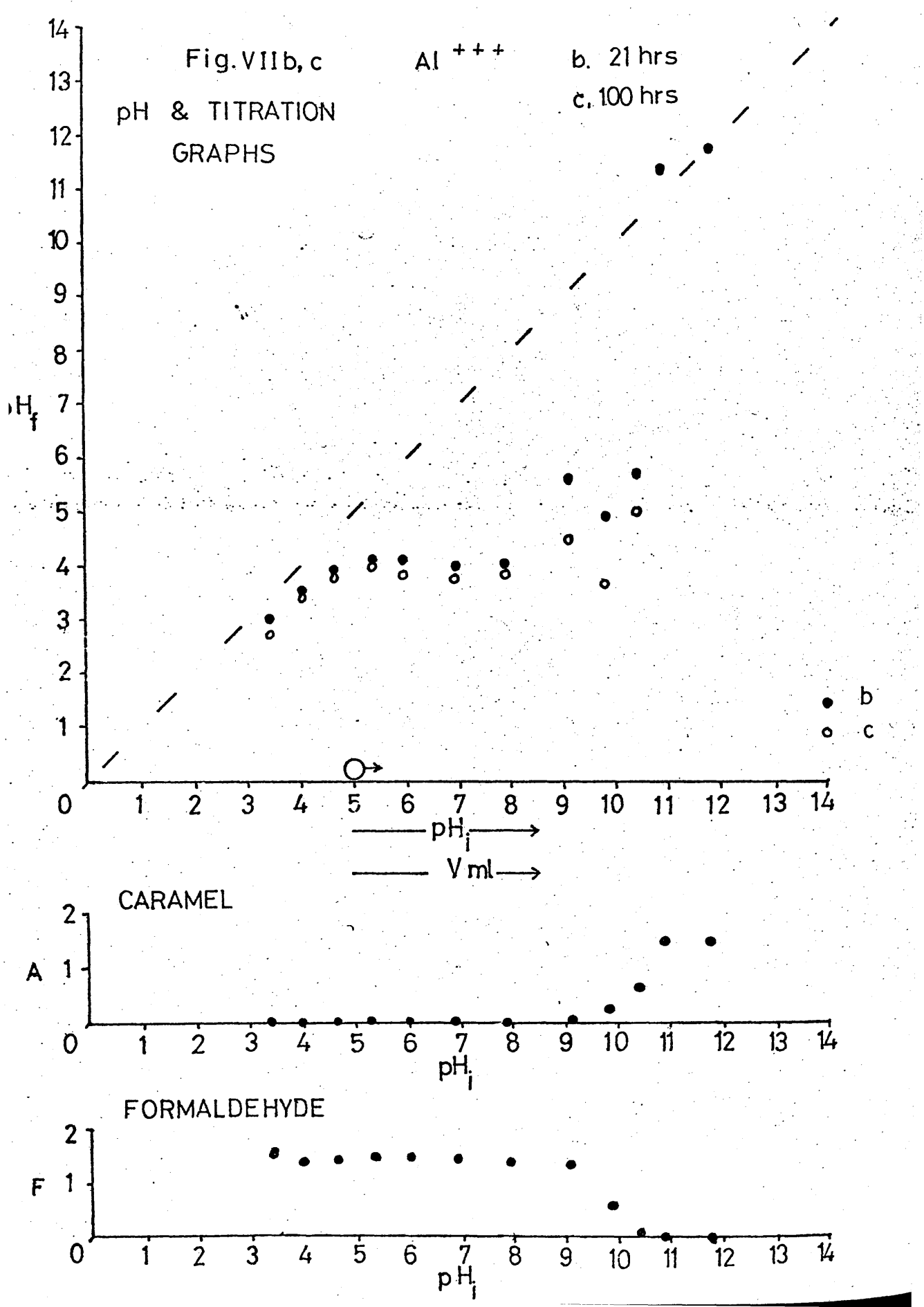


Fig. VIIb, c
Al +++
b. 21 hrs
c. 100 hrs
pH & TITRATION GRAPHS
pHf
pHi
V ml
b
c
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

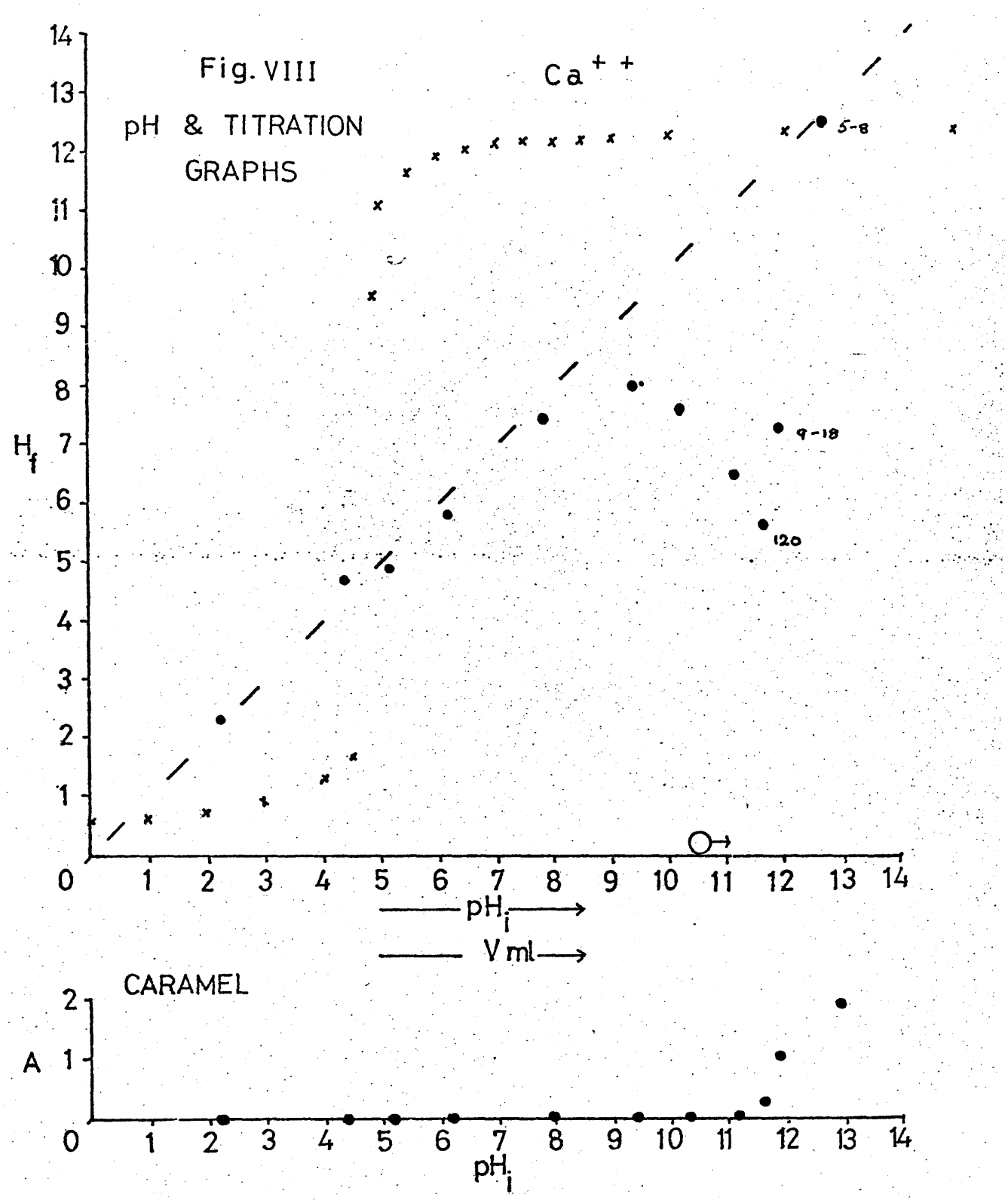


Fig. VIII
pH & TITRATION GRAPHS
Ca++
5-8
9-18
120
Hf
pHi
V ml
CARAMEL
A
pHi

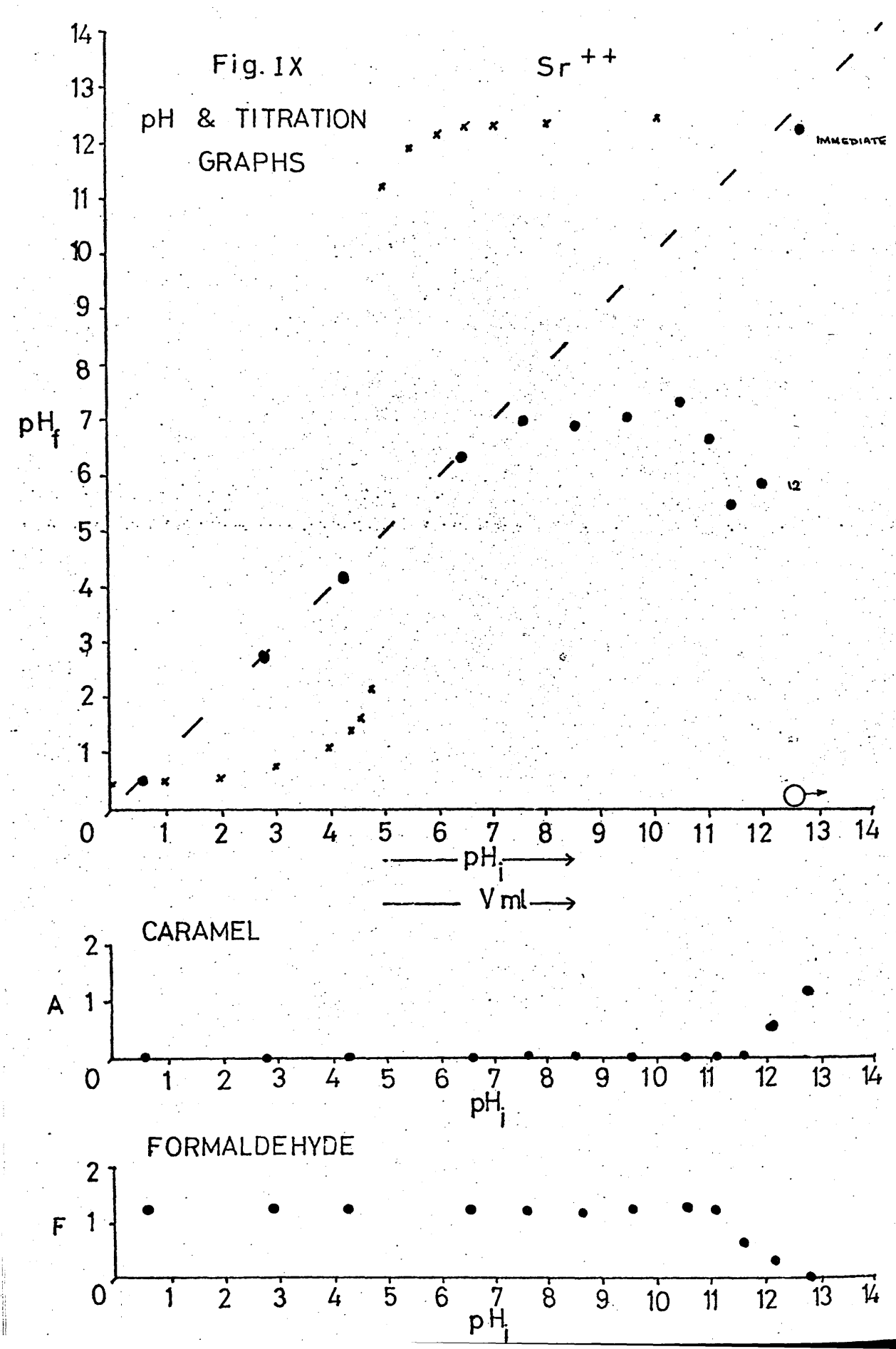


Fig. IX
pH & TITRATION GRAPHS
Sr^{++}
IMMEDIATE
12
pH_f
pH_i
V ml
CARAMEL
A
pH_i
FORMALDEHYDE
F
pH_i

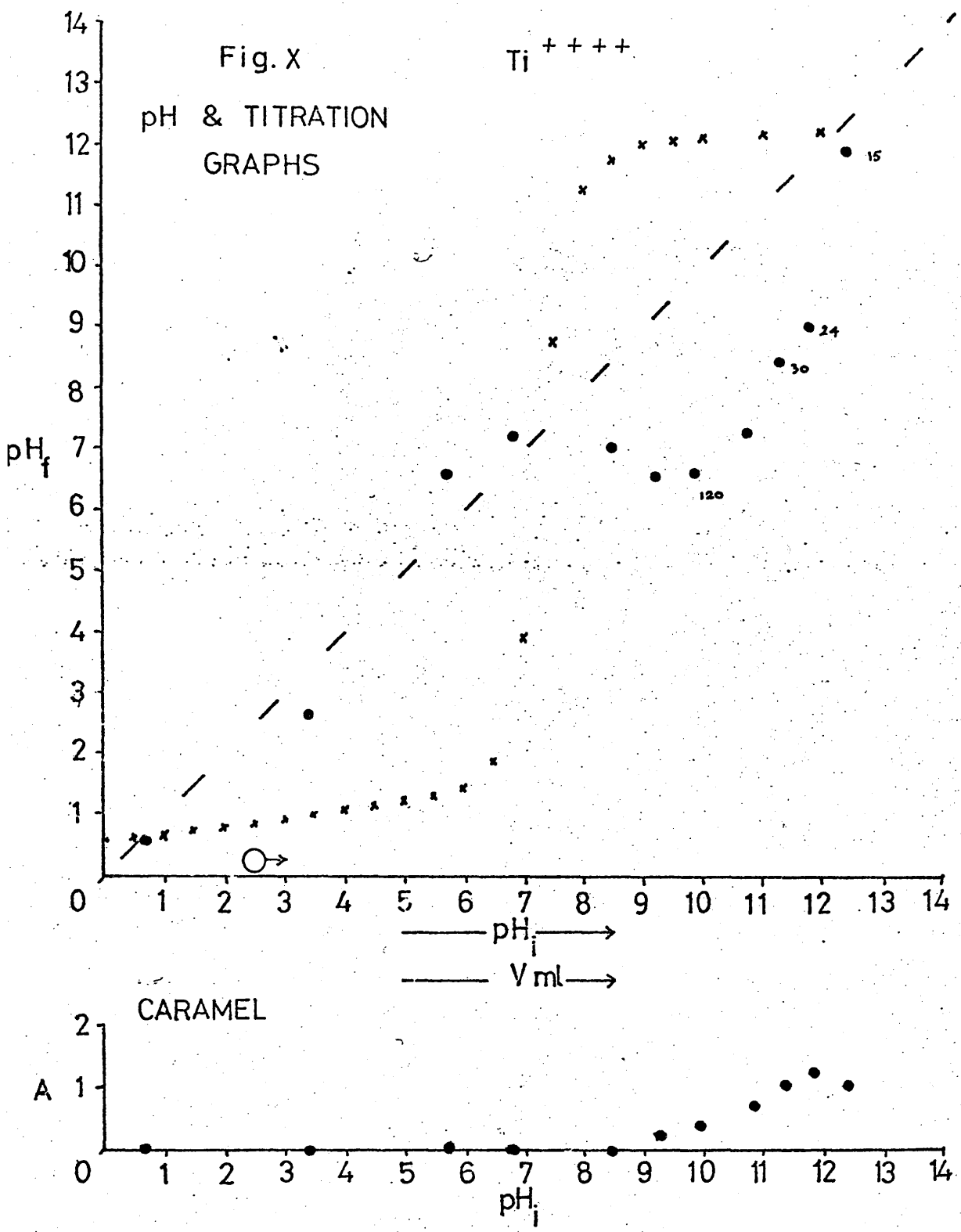


Fig. X
pH & TITRATION GRAPHS
Ti^{++++}
15
24
30
120
pH_f
pH_i
V ml
CARAMEL
A
pH_i

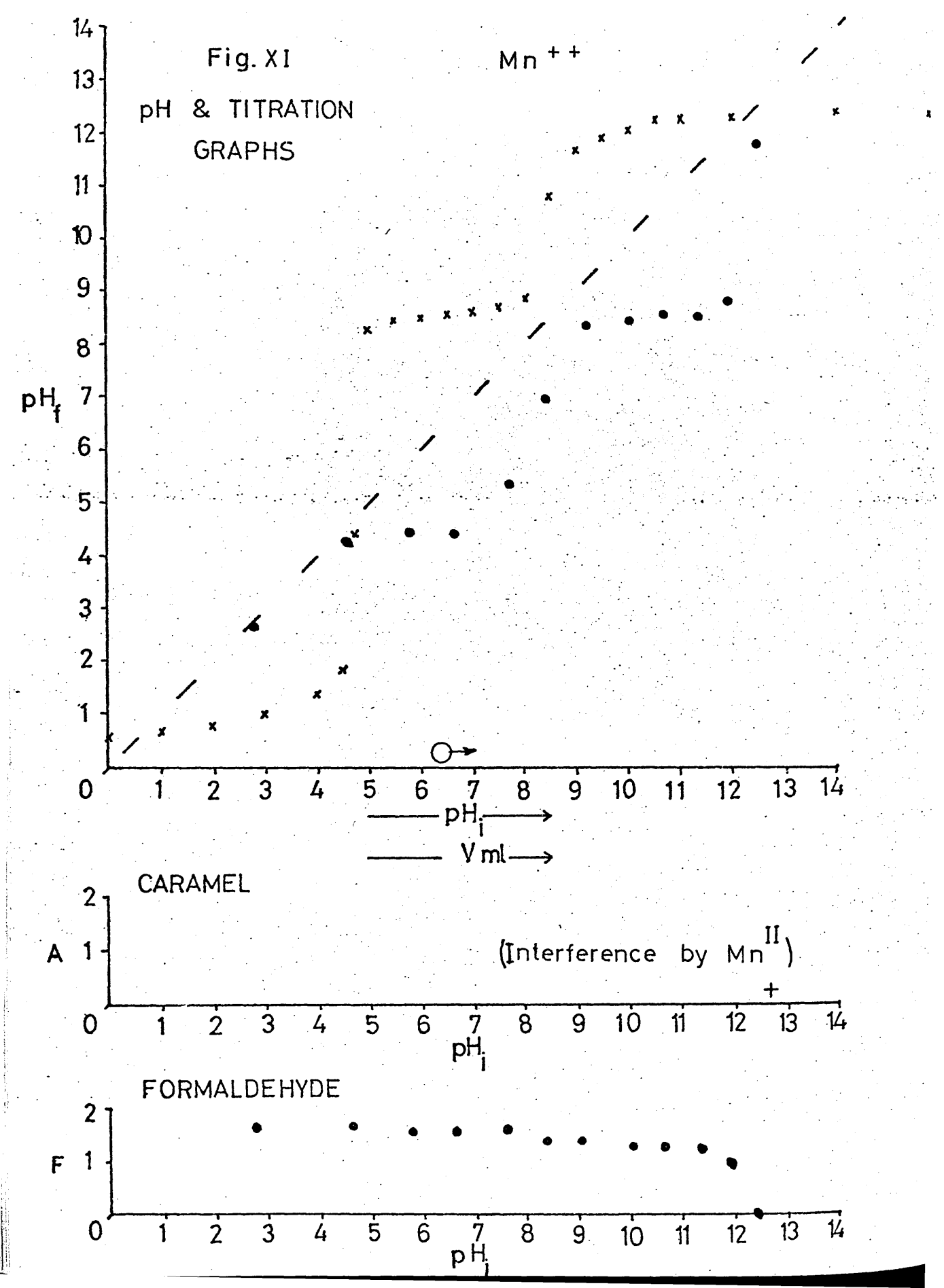


Fig. XI
pH & TITRATION GRAPHS
Mn++
pHf
pHi
V ml
CARAMEL
A
(Interference by Mn++)
pHi
FORMALDEHYDE
F
pHi

Magnesium hydroxide exhibits a differently shaped curve (Fig. VI). There are two plateaux. The lower is due to the Cannizzaro "switch off" and the higher to the bufferring of the magnesium hydroxide. The solution is heterogeneous above pH 5. Formose is not made below pH_i 11.5.

Aluminium hydroxide exhibits caramel formation down to pH 10, much lower than the sodium, lithium or magnesium values. The Cannizzaro "switch off" value is masked by an aluminium hydroxide buffer region and is at pH 4 or lower. (Fig. VIIa.) Prolonged refluxing of these solutions did not reduce the Cannizzaro plateau significantly, but did cause a reduction between pH_i 9 and 11, probably because of caramelisation of the sugars, or a cross-Cannizzaro reaction. (Fig. VIIb,c.)

Calcium hydroxide causes sugar formation above pH 11.5 (Fig. VIII) under these conditions, but the Cannizzaro reaction is not clearly defined. This may be due to a reduced rate of the latter reaction.

Strontium ions form sugars above pH_i 11.5, similar values to magnesium and calcium. The browning times with strontium hydroxide were much more rapid than with the other alkaline earths, indeed a brown colour was noticed in the flask at pH_i 12.6 within one minute of the flask being flaced on the sandbath. (Fig. IX.)

Some transition metals can catalyse the formose reaction. Titanium hydroxide produces sugars above pH 8.5, the lowest value yet for a metal ion. (Fig. X.), but the Cannizzaro "switch off" pH is not clear.

Moving across the row of the transition metals, we come to manganese. The colour of the manganese solution masks the measurement of caramel by the usual means, however the odour of caramel and a slight hint of brown was noticed in the solution of initial pH 12.4. Again a buffer plateau is found. The Cannizzaro "switch off" is ca. pH 4. (Fig. XI.)

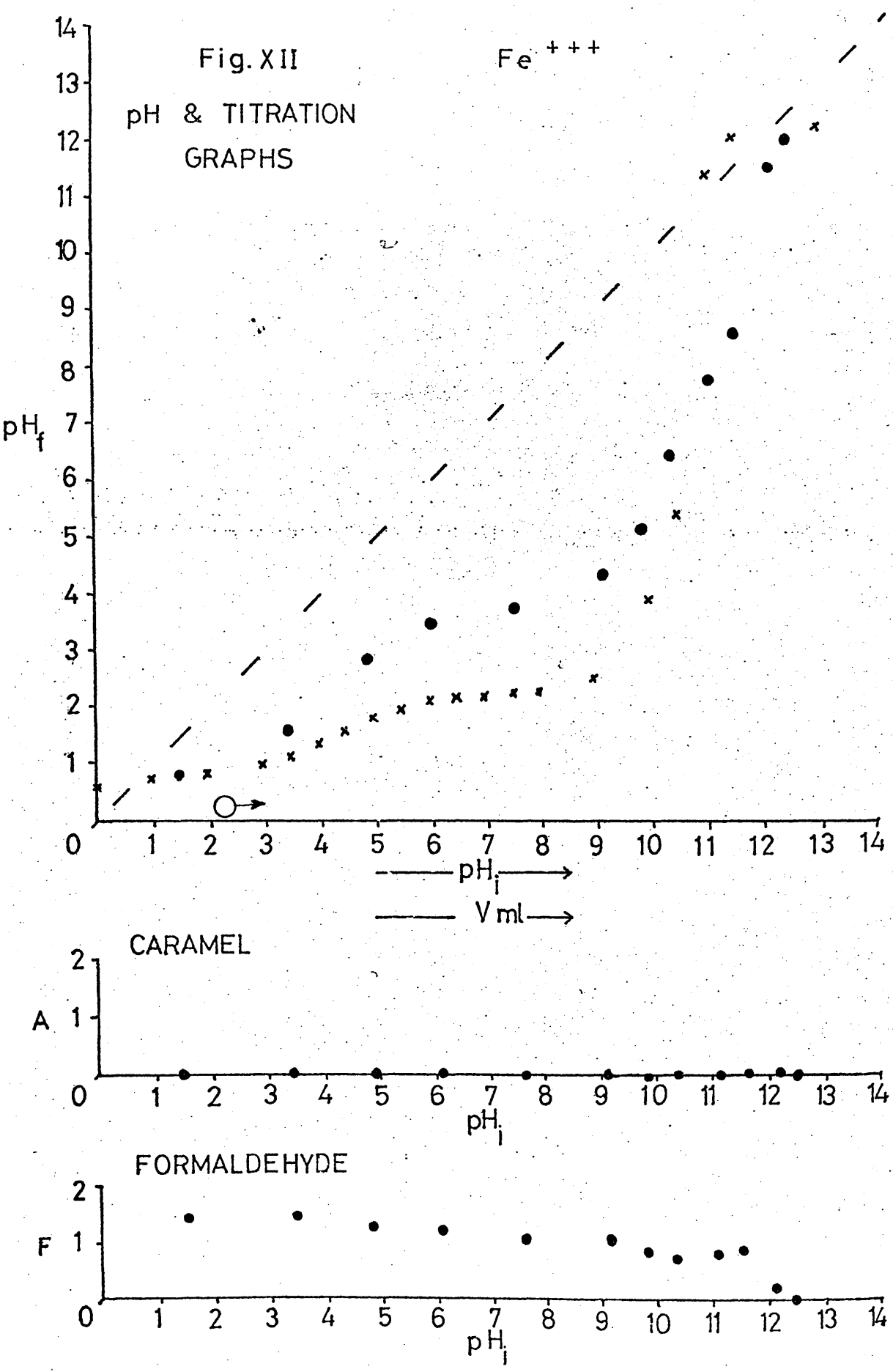


Fig. XII
pH & TITRATION GRAPHS
Fe^{+++}
pH_f
pH_i
V ml
CARAMEL
A
pH_i
FORMALDEHYDE
F
pH_i

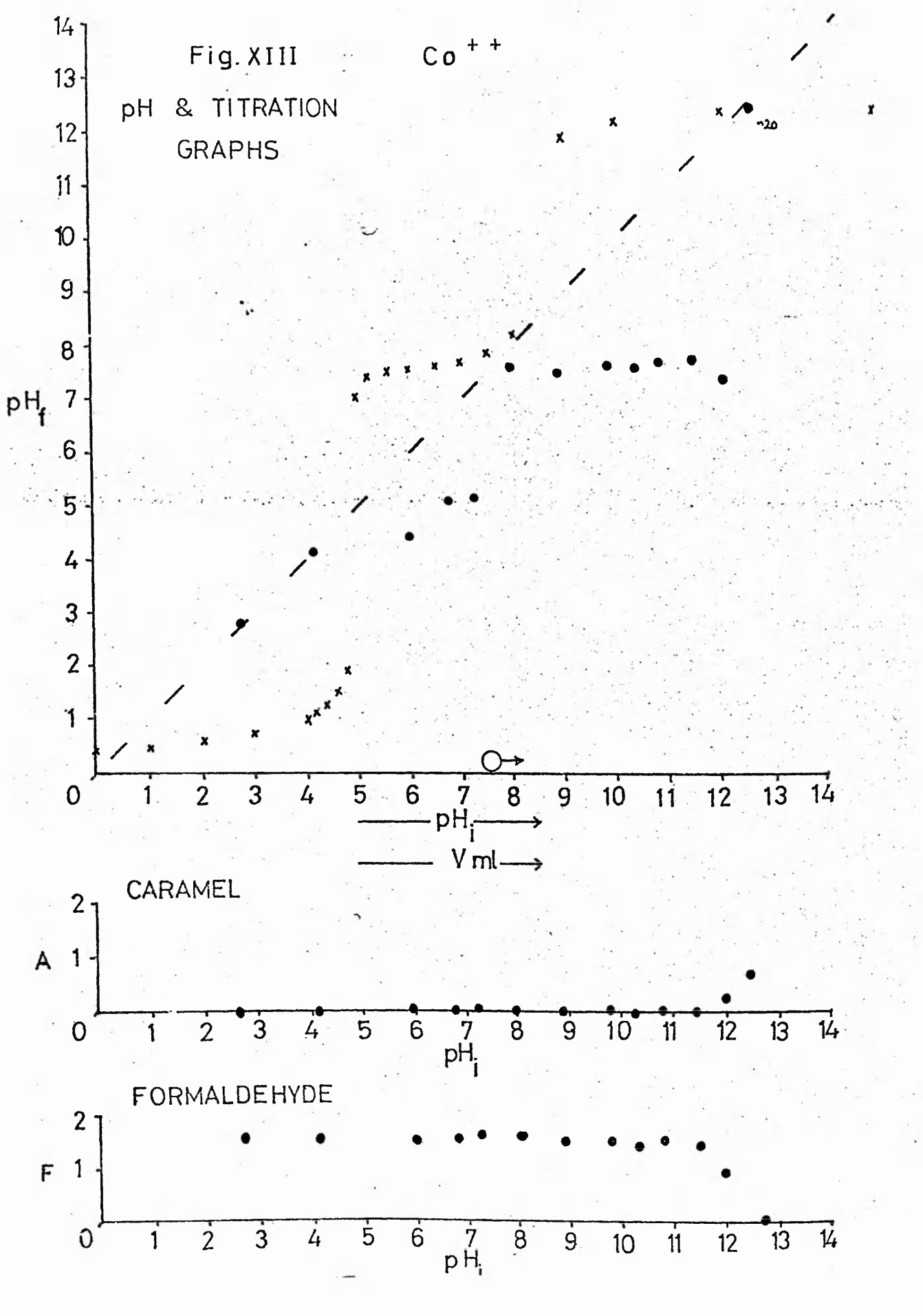


Fig. XIII
Co ++
pH & TITRATION
GRAPHS
pH_f
pH_i
V ml
CARAMEL
A
pH_i
FORMALDEHYDE
F
pH_i

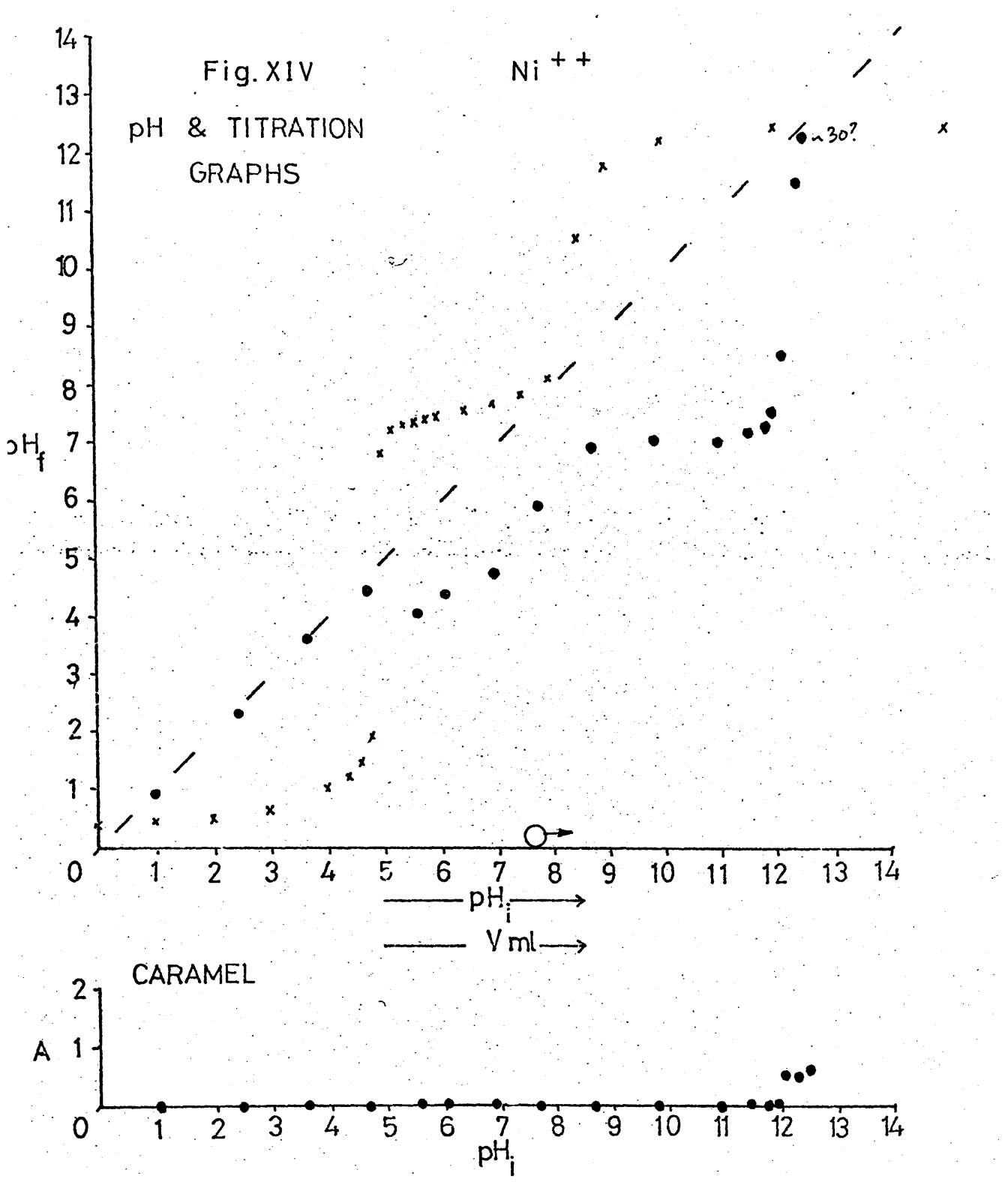


Fig. XIV
pH & TITRATION GRAPHS
Ni ++
pH_f
pH_i
V ml
CARAMEL
A
pH_i

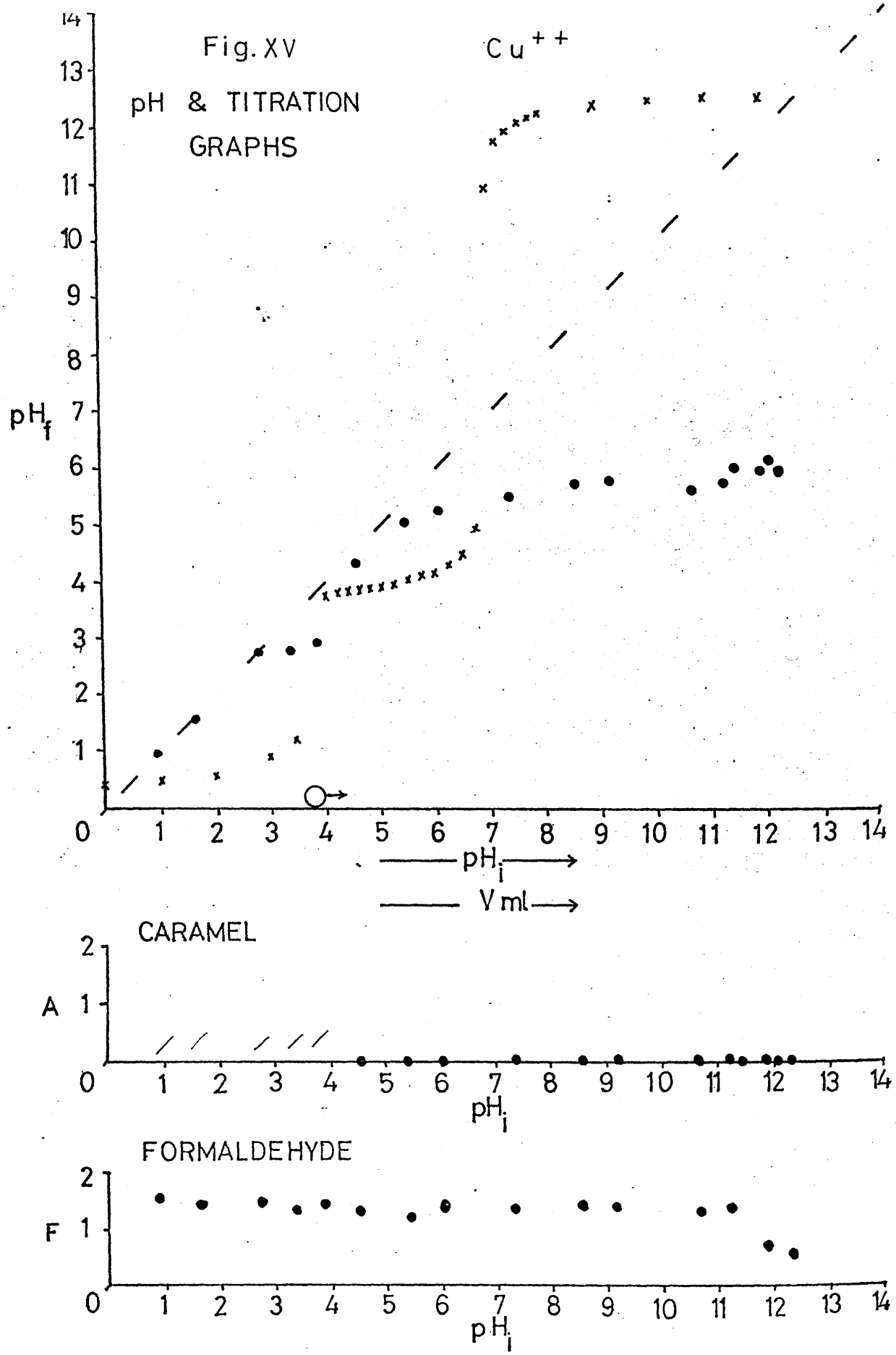


Fig. XV
pH & TITRATION GRAPHS
Cu++
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

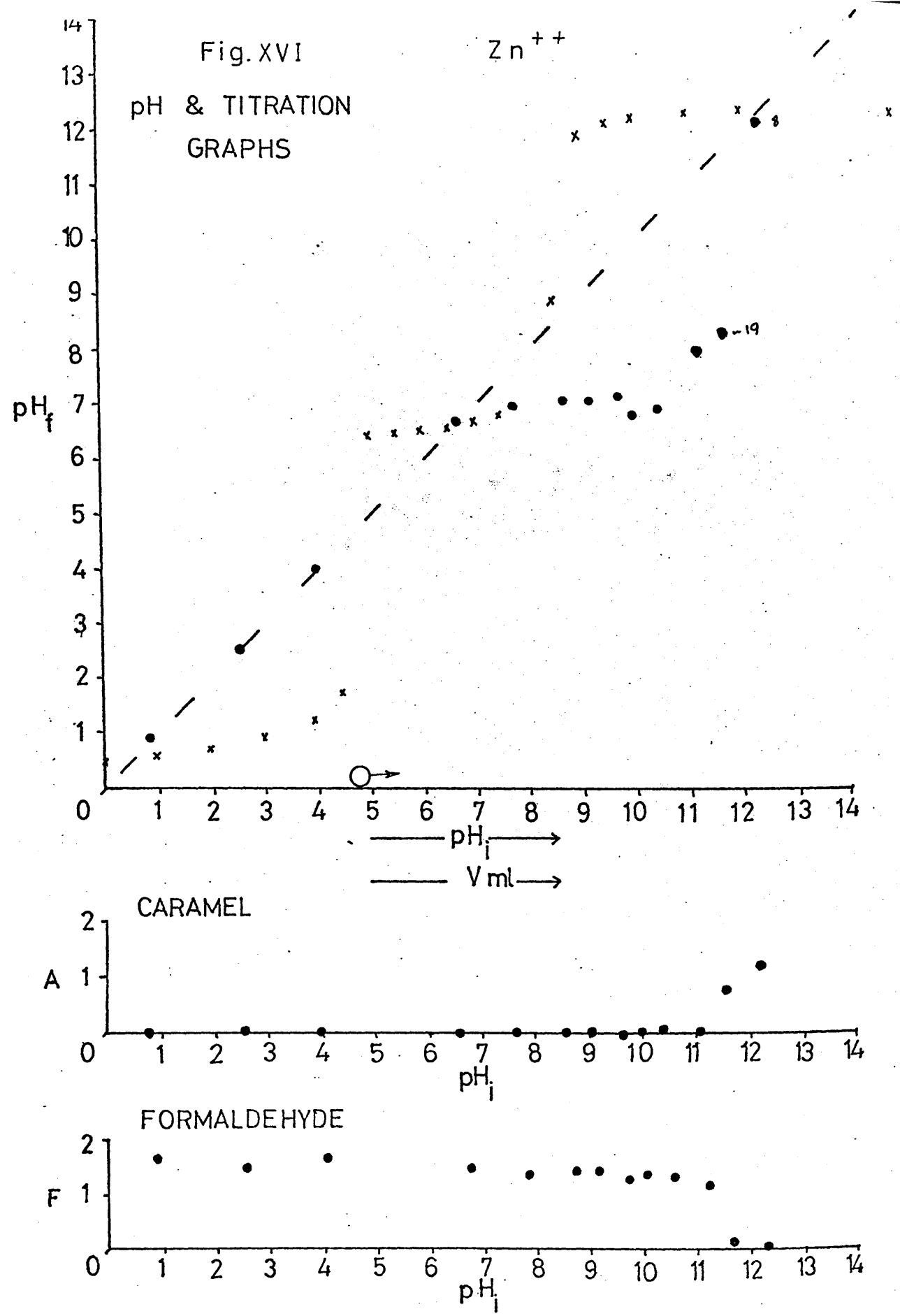
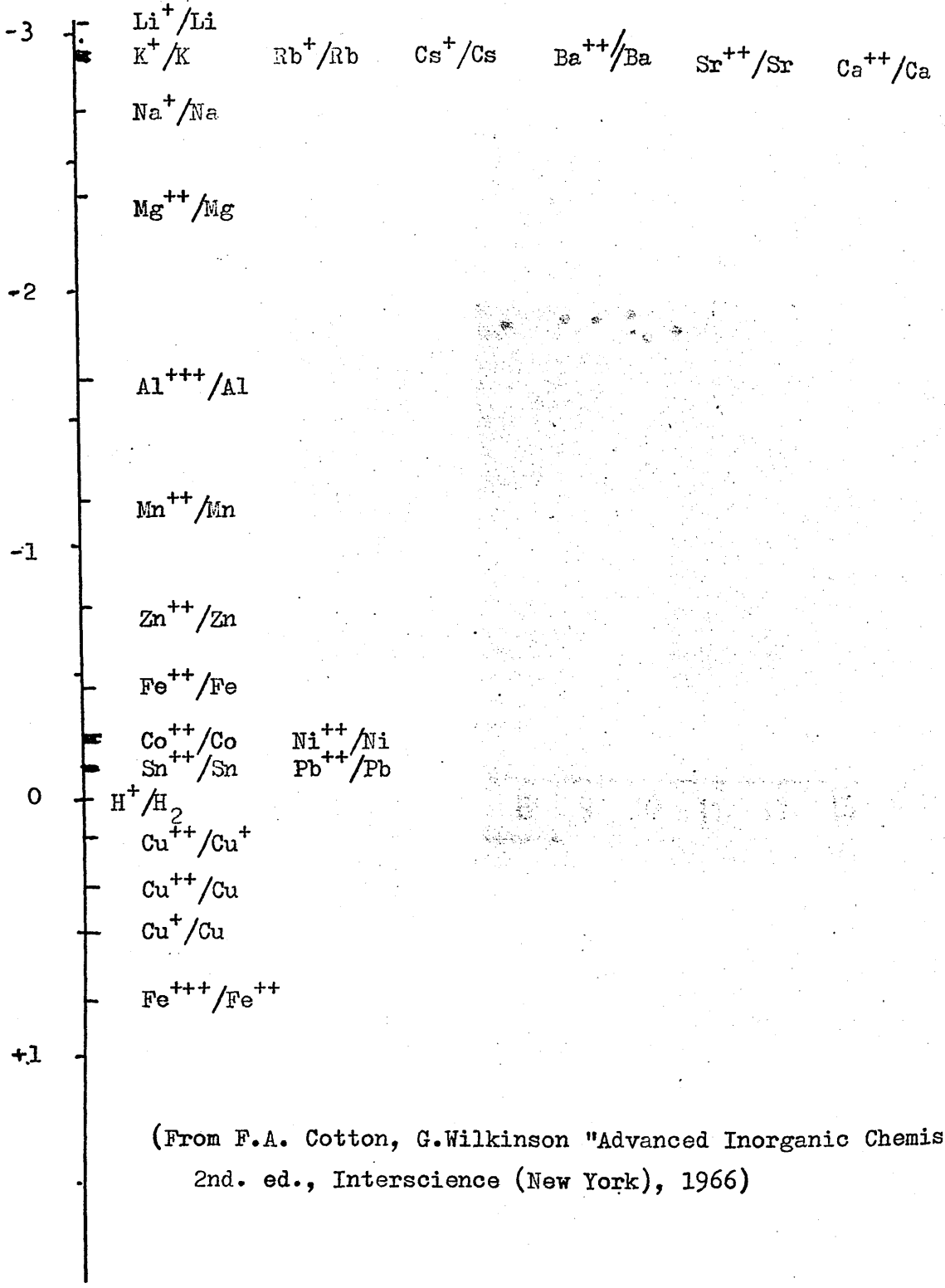


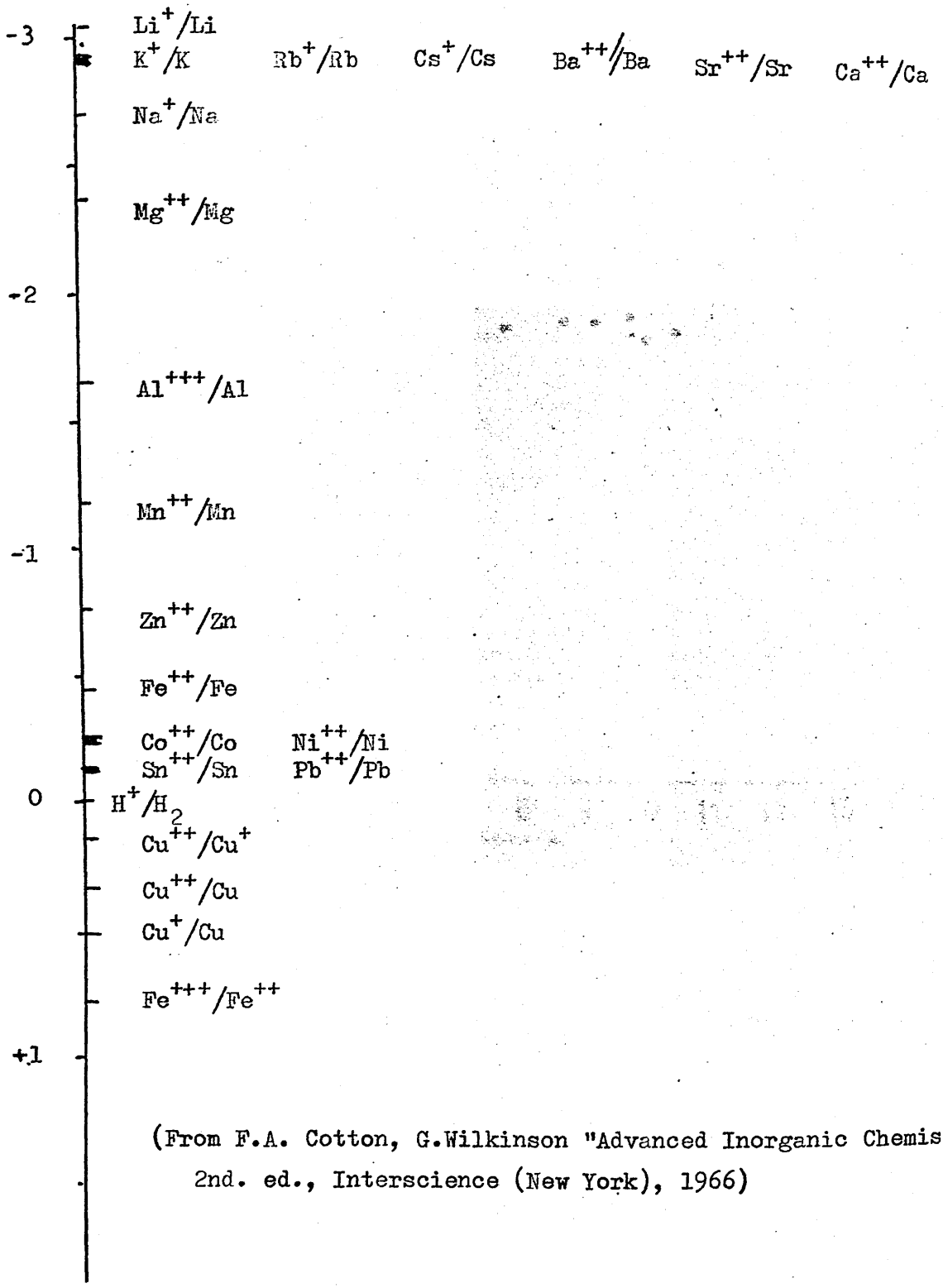
Fig. XVI
Zn++
pH & TITRATION GRAPHS
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

TABLE B. REDUCTION POTENTIALS



(From F.A. Cotton, G.Wilkinson "Advanced Inorganic Chemistry" 2nd. ed., Interscience (New York), 1966)

TABLE B. REDUCTION POTENTIALS



(From F.A. Cotton, G.Wilkinson "Advanced Inorganic Chemistry" 2nd. ed., Interscience (New York), 1966)

TABLE C. FORMOSE "SWITCH ON" AND CANNIZZARO "SWITCH OFF" pHs.

CATION	FORMOSE "SWITCH ON"	CANNIZZARO "SWITCH OFF"
Li^{+}	11.7	6.7
Na^{+}	11.5	6.0 - 6.5
Me_4N^{+}	7.5	-
Mg^{++}	> 11.5	5.2
Ca^{++}	11.5	?
Sr^{++}	11.5	7?
Al^{+++}	10.0	3.9
Ti^{++++}	8.5 - 9.C	?
Mn^{++}	~ 12	~ 4
Fe^{+++}	-	-
Co^{++}	11.9	4.5 - 5.0
Ni^{++}	~ 12	4.0 - 4.5
Cu^{++}	-	(5.5 - 6.0)
Zn^{++}	11.4	7.0

TABLE D

CATIONS INVOLVED IN "SWITCH-ON" INVESTIGATIONS

H																He
Li^{I}	Be											B	C	N	O	F	Ne
Na^{I}	Mg^{II}											Al^{III}	Si	P	S	Cl	Ar
K	Ca^{II}	Sc	Ti^{IV}	V	Cr	Mn^{II}	Fe^{III}	Co^{II}	Ni^{II}	Cu^{II}	Zn^{II}	Ga	Ge	As	Se	Br	Kr
Rb	Sr^{II}	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	+	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	++															

+	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
++	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md		

Me_4N^+

Ferric ions can be reduced by formaldehyde, resulting in formic acid. As we can see (Fig. XII) acid is produced in almost every case and no sugar was found. Again the buffer ing effect is observed. A list of reduction potentials of the various ions that have been examined in this study will be found in Table B.

Cobalt(II) was the next ion to be examined. Formose was produced at pH values higher than 11.9. The curve (Fig. XIII) is similar to that which we obtained from magnesium and nickel(II) (Fig. XIV).

Copper(II) is reduced by formaldehyde and no sugars are formed (Fig. XV).

Zinc hydroxide is the last compound that we shall examine. Sugars are formed above pH 11.2. (Fig. XVI.) The Cannizzaro and buffer plateaux coincide.

The various "switch on" and "off" values have been tabulated. They are recorded in Table C, Individual cations are identified in the periodic table (Table D).

The cations may be divided into two immediate categories: those that catalyse the formose reaction and those that do not, because they oxidise the formaldehyde. Of the former category the higher the charge on the ion, the lower is the pH at which formose is formed. The principal exception to this is the tetramethyl-ammonium ion which may not have any effect other than supplying supplies of unco-ordinated hydroxide ions. The formose values for the alkali metals and the alkaline earths are approximately the same: those for some divalent transition metals seem to be fractionally higher.

It is difficult to see any definite trends in the Cannizzaro "switch off" values, although the pH does fall as the charge on the ion rises, which is again what we would expect. We might have expected not to find any Cannizzaro "switch off" values greater

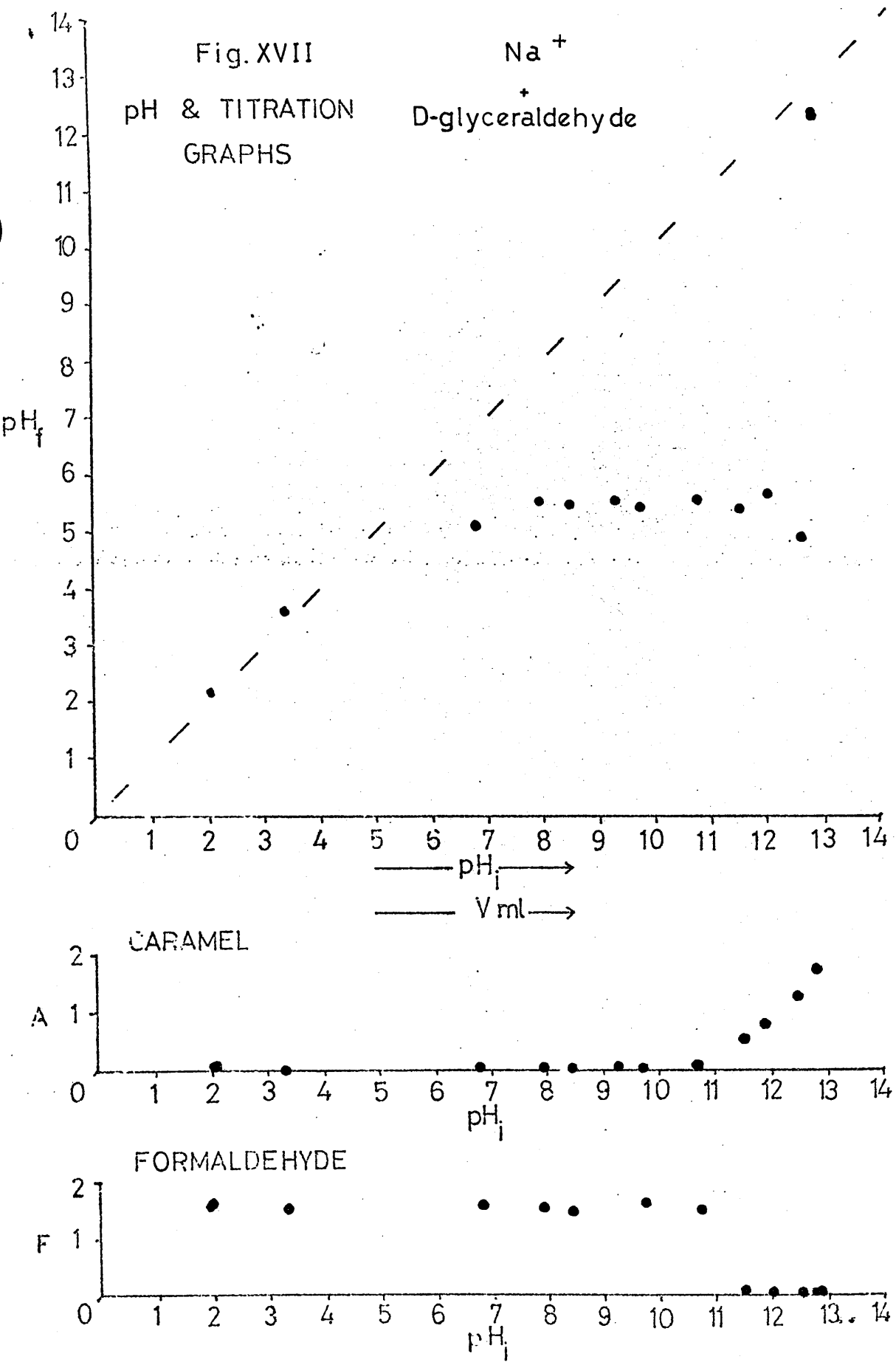


Fig. XVII
pH & TITRATION GRAPHS
Na +
+
D-glyceraldehyde
pH f
pH i
V ml
CARAMEL
A
pH i
FORMALDEHYDE
F
pH i

than 6.0 - 6.5, the level for sodium ions. (Sodium hydroxide was added to solutions of the chlorides of metals outwith Group I.) Zinc ions have a value of approximately 7, which is on the border of experimental error between the sodium and zinc values. In general the lowest Cannizzaro figures are obtained with transition metals. It seems from this that co-ordination for the Cannizzaro reaction is necessary, but that the formose reaction need not rely on it.

Neutral aluminium oxide, exerting a pH of approximately 7½ in aqueous solution can catalyse the formose reaction, yet the "switch on" value for aluminium hydroxide is ca. 10.0. The reason for this discrepancy may be due to local extremes of pH on the alumina surface.

The Effect of an Accelerator on the pH Curve for Sodium Ions.

The addition of a formose reaction accelerator, it is generally thought, provides a way of starting the aldol reactions by circumventing the primary reaction. We might expect that the formose reaction (aldol part only) would be able to occur at a lower pH, and so should see a movement of the formose "switch on" value to lower pH.

D-Glyceraldehyde was added to the sodium hydroxide system and the formose reaction started at half a unit lower. (Figs. I, XVII). The Cannizzaro final pH also fell, by about one unit. Cross-Cannizzaro reactions with glyceraldehyde may have caused this different minimum. (If not, and the glyceraldehyde was having to accept a passive, co-ordinating role, as in Pfeil's complex, we should have to explain why the presence of the sugar would ease hydride transfer from a neighbouring formaldehyde molecule.) The possibility of a cross-Cannizzaro reaction in Pfeil's complex should not be overlooked.

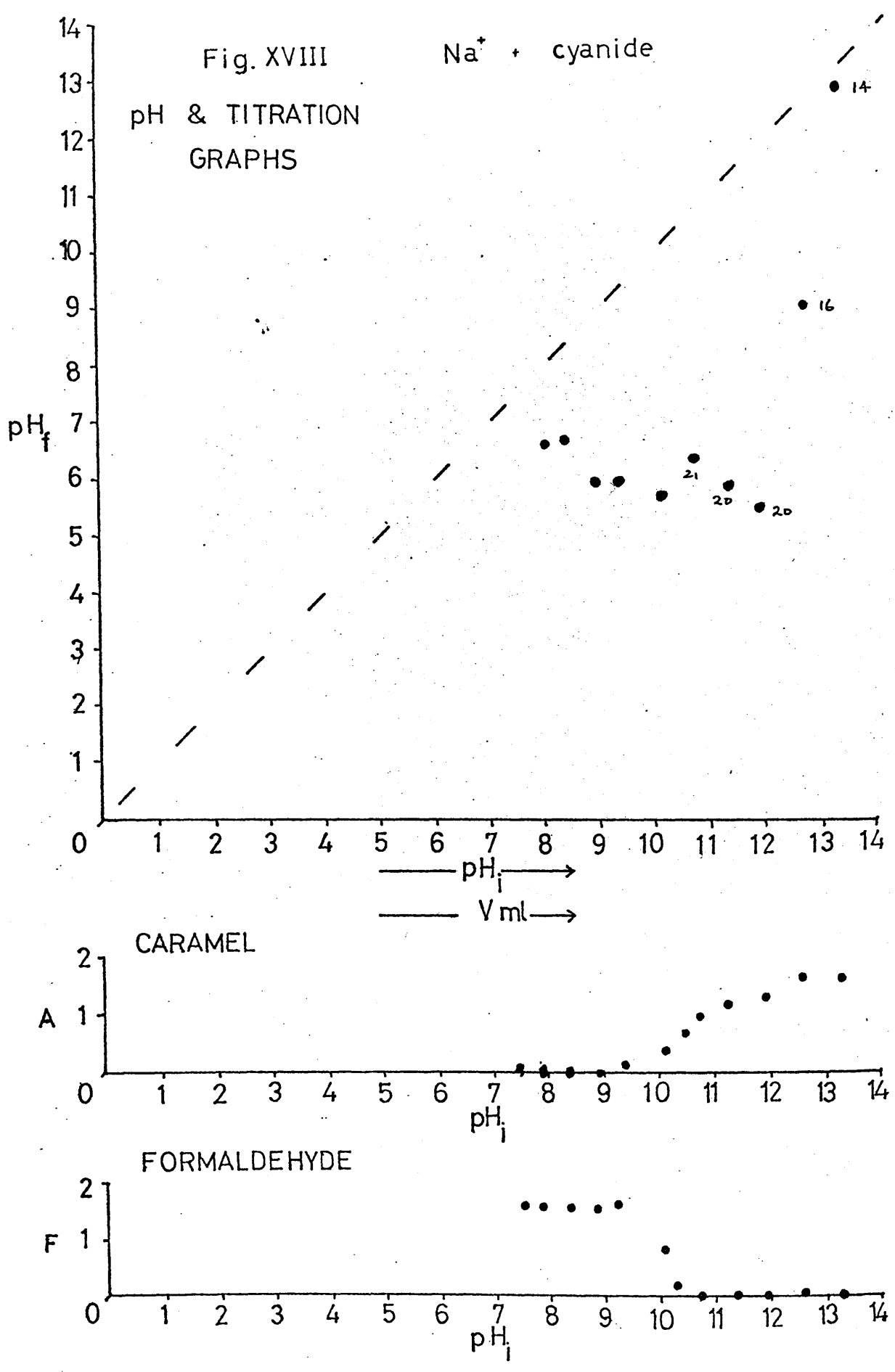


Fig. XVIII
pH & TITRATION GRAPHS
Na+ + cyanide
pHf
pHi
V ml
CARAMEL
A
pHi
FORMALDEHYDE
F
pHi

The Effect of Cyanide Ions on the pH Curve for Sodium Ions.

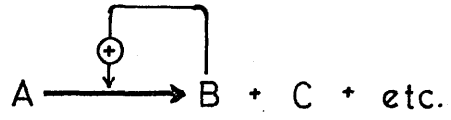
In Chapter 2 the effect of cyanide on the alumina catalysed formose reaction was studied. It has no effect. This was however in a heterogeneous system. What effect is there in a homogeneous system, more specifically what does cyanide do to the formose "switch on" value in the sodium hydroxide system?

The addition of cyanide reduced the minimum value for sugar formation to pH 9.5 (Fig. XVIII). The Cannizzaro value is approximately, similar to the value in the absence of cyanide but here sugar caramelisation may account for some of the acid.

The implication of this finding is that the benzoin-type of acyloin condesation is possible with formaldehyde. This gives further support to the theory in which formaldehyde need not be co-ordinated to a metal ion to undergo the formose reaction. We should remember that cyanide could be providing yet another pathway open to the primary reaction: it need not necessarily be a confirmation of the hydrate mechanism.

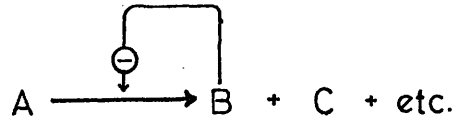
Autocatalytic and Autoinhibitive Reactions.

An autocatalytic reaction is one in which one or more of the products enhance their own formation. An example is the well-known acid-catalysed hydrolysis of an ester. A further example is the second stage of the formose reaction (see Breslow's mechanism, Chapter 1). Schematically we may draw the process as follows:

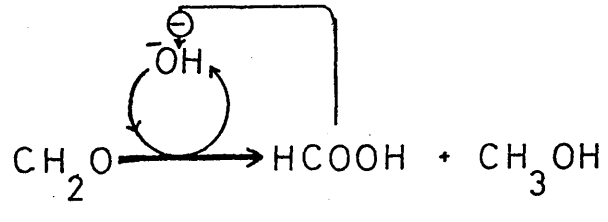


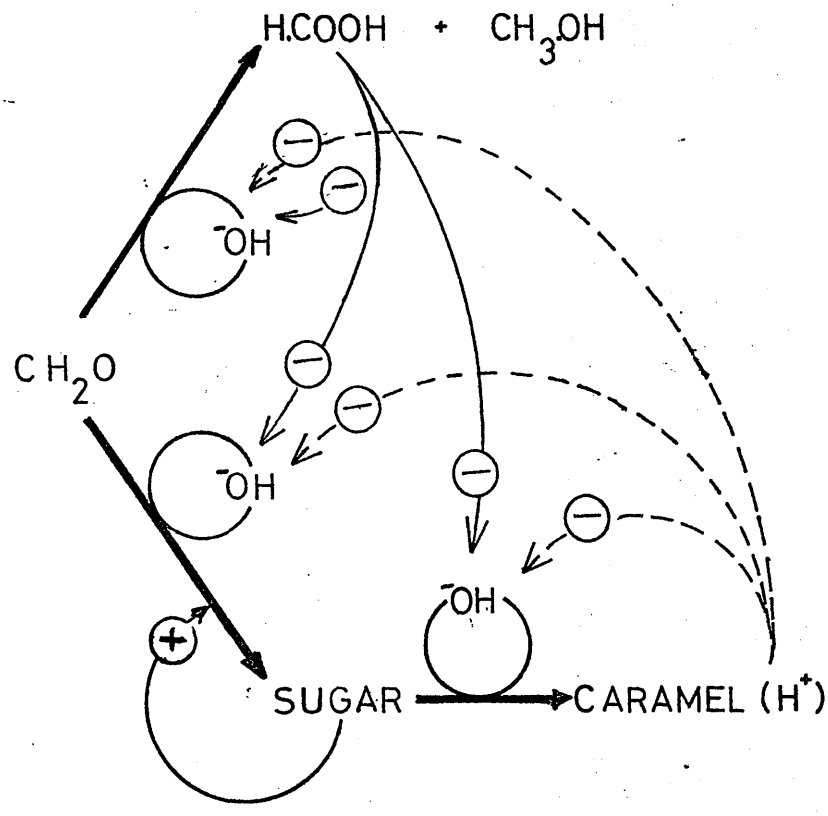
We shall use "+" for a rate enhancing effect. For this kind of reaction to cease, the supply of A must stop, the reaction will continue until all of A has been consumed, in a closed system and in the absence of side reactions which would alter the autocatalysis.

Reactions in which the products antagonise their own formation are also known. In the above scheme we replace the "+" with a "−".

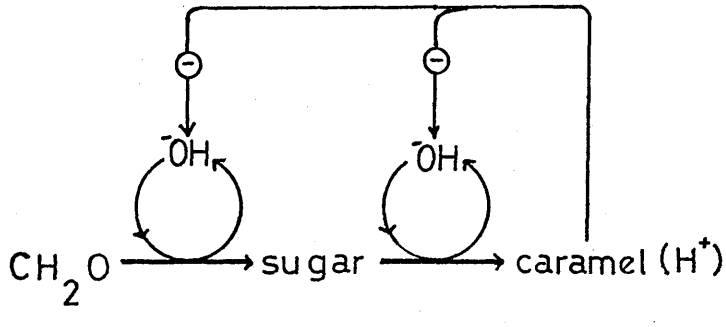


An example of this kind is the Cannizzaro reaction, which we have just observed. Here the acid produced acts not on the formaldehyde, but on the catalyst (the hydroxide part). Also the reaction may stop before the supply of reagent is exhausted.




H.COOH + CH3OH
CH2O
OH
OH
OH
SUGAR
CARAMEL (H+)

The formose reaction may be autoinhibitory. Caramel is produced. This reduces the pH and slows the formose reaction down. In a closed system the rate of the formose reaction will not be markedly affected by this as the rate of destruction of the sugars is slower. (The high initial pHs in the sodium hydroxide system where sugar has been formed, are not markedly reduced by caramelisation.) In a system where formaldehyde is being constantly added and where the caramel must accumulate, the autoinhibition will be observed, although there will be a considerable delay. A small measure of the Cannizzaro reaction will also contribute to this.



If we combine the Cannizzaro and formose reactions into the "formose system", then there can be quite a complicated system of controls. (Pfeil's inhibition of the formose reaction by large amounts of glucose was not confirmed in Chapter 2, and has not been entered into the diagram.) The formose system is shown on the opposite page. It is clear that the rates of the various processes will determine the outcome of a given set of conditions.

Discussion.

The Method of Preparation of the Solutions at Various pHs.

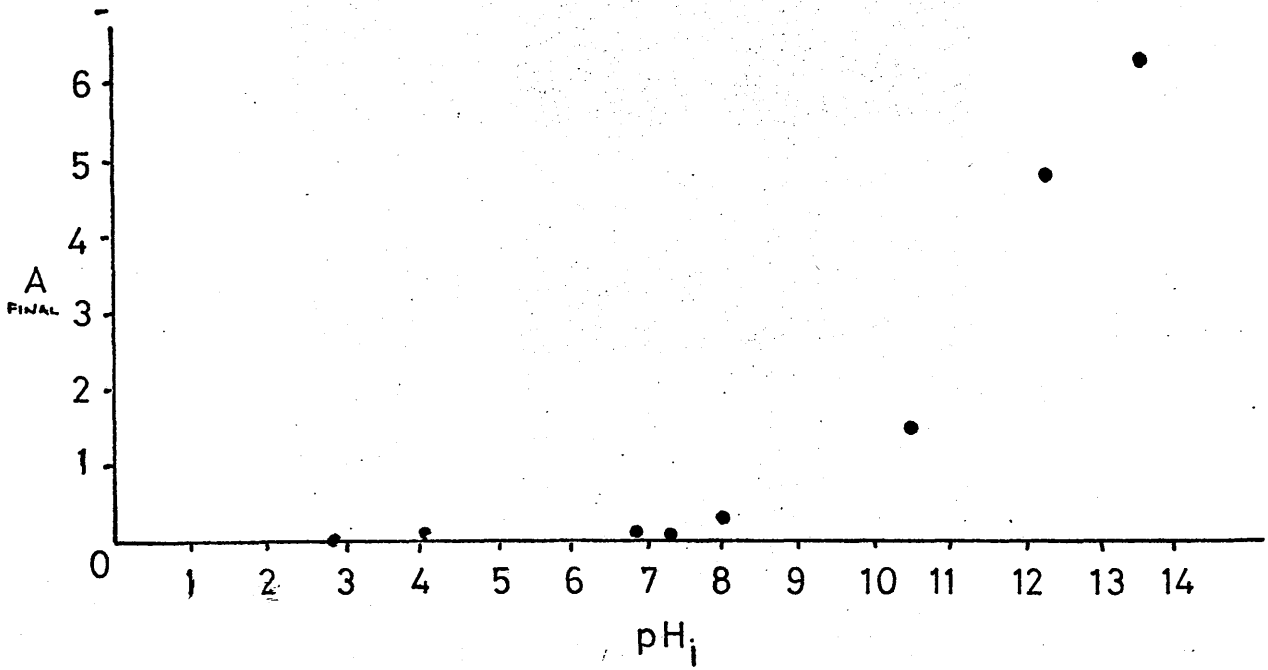
In the last Chapter we saw that the response of aluminium oxide to added acid and alkali was slow, and that the time for hydration of the alumina was long. For this reason we chose to examine the homogeneous system first. Sodium, lithium and tetramethylammonium hydroxides were all available for the experiment, and hydrochloric acid was added to attain lower values of pH. Chloride was chosen as it is likely to have less effect, if indeed any, than nitrate or phosphate.

The hydroxides of many other metals are insoluble, so we are faced with the same problem as before. The chlorides of all the cations used,were soluble in water or acid. For practical reasons, mainly the time that was taken for a given solution to reach equilibrium, solutions of the chlorides were made basic by the addition of sodium hydroxide. This has the disadvantage that extraneous (sodium) ions are being added to the system under study, but has the advantage that the hydroxides could be made under standard conditions, rather than relying on samples of hydroxide which had been prepared some time ago, and which might have contained unknown amounts of oxide and carbonate.

In principle it is possible to study the effects of lead barium,etc, under these conditions, but as their chlorides are rather insoluble, the time taken to reach equilibrium in each flask would have been considerable.

Fig. XIX

GLUCOSE CARAMELISATION AT DIFFERENT pHs.



A is absorbance at maximum 225 - 275nm.

ULTRA-VIOLET ABSORPTION OF CARAMEL

Many of the solutions under study turned brown or yellow, when sugar had been produced. The ultra-violet spectrum of this caramel showed a broad absorbance with a maximum between 225 and 275nm. In some solutions which were clear to the eye an absorption was found in this region: the pH of these solutions was high and was immediately below solutions which were seen to be brown or yellow.

It was hoped that the absorbance measurement could have been takn taken as a measure of the amount of sugar formed. In order to confirm this, glucose solutions at various pHs were refluxed for five hours and the absorbances then measured. The graph of absorbance against pH is given in Fig. XIX (opposite). Clearly our earlier hope was not borne out. The absorbance could be used for a quantitative estimate of caramel in a number of solutions of the same pH, but not of differing values.
The appearance of an absorption should therefore be taken only as an indication of the presence of sugar degradation products.
It is suitable for use above pH 7.5

The symbol "A" represents the absorbance on the graphs.

THE DETERMINATION OF FORMALDEHYDE

The suitablilty of the chromotropic acid method for the determination of formaldehyde was confirmed by Weiss.[133] It was previously known that glyceraldehyde, arabinose, fructose, and furfural did not interfere with the formaldehyde estimation, although they did give a yellow colour. Interference by acetaldehyde, and β-hydroxypropionaldehyde could be overcome by the use of excess acid. Other compounds, such as methanol, formic acid, acetic acid, acetone and glycerol, either had no reaction or a slight one.[215]

The quantitative determination of formaldehyde has been carried out in most of these experiments but in many of the experiments in other chapters a qualitative determination proved sufficient. The kinetics of the formose reaction were discussed in the first two chapters. When sugar had been formed, in almost every case, no formaldehyde remained after five hours. The measurement of formaldehyde in a system where it need only be known whether the formose reaction has occurred to any significant extent, can be done qualitatively. The accompanying Cannizzaro reaction can reduce the formaldehyde content to some small extent but did not consume the formaldehyde completely in any of the experiments in this thesis. Where the formose reaction is under way at a very slow rate i.e. where formaldehyde remains after five hours, the qualitative test will show a lesser intensity of formaldehyde. (The qualitative determination of the aldehyde was used in conjunction with other observations e.g. colour and odour of solution, in making an assessment of whether the formose reaction has occurred.)

CHROMOTROPIC ACID METHOD OF FORMALDEHYDE DETERMINATION

A solution of 1.0g. sodium chromotropate* in 100ml. distilled water was prepared. To this 450ml 65% sulphuric acid solution were slowly added. Much heat was evolved in the initial stages of this process and the flask was cooled in ice. The solution was swirled occasionally during the next half hour and then filtered through glass paper. (Ordinary fliter paper chars on contact with the sulphuric acid.) The solution was now ready for use. The solution was stored at 0° when not in use, and remained usable state for several weeks.

0.05ml. aliquot of the diluted solution (see expt. I) was added to 7.0ml chromotropic acid reagent. After mixing the solution was heated at 100° for 15 minutes. In the presence of formaldehyde a deep blue colour developed. The absorbance of this solution was measured at 570—580nm. in a 5 or 10mm quartz cell.

The symbol "F" is used for the absorbance of the solution. (10mm. cells.)

* Hopkin and Williams, Ltd.

Methods.

Preparation of Solutions of Hydroxides or Chlorides of the Metals.

0.39M solutions of sodium, lithium and tetramethylammonium hydroxides (BDH) were prepared in distilled water. 0.39M solutions of the chlorides of magnesium, calcium, strontium, manganese(II), iron(III), cobalt(II), nickel(II), copper(II) and zinc(II) (BDH) prepared in like manner. Aluminium (Fluka) and titanium(IV) (BDH) chloride were also added to water to give 0.39M solutions. These materials reacted violently with the water, so great care was taken to add them slowly. Where necessary, hydrochloric acid ('AnalaR', BDH) was added to the solutions to dissolve any precipitate, before they were diluted to the required strength.

Titration of the Solutions.

5.00ml. portions of sodium, lithium and tetramethylammonium hydroxide were titrated with N/1 hydrochloric acid (Concentrated Volumetric Solution, BDH). The pH of the solution was recorded, and stirring effected with a magnetised rod.

5.00ml. portions of the solutions of the chlorides were placed in flasks with 5.0ml portions of hydrochloric acid (ca. N/1, "AnalaR"). N/1 sodium hydroxide solution (Concentrated Volumetric Solution, BDH) was added and the pH followed.

The Experiments.

I NaOH - HCl

10.0ml portions of 0.39M sodium hydroxide solution were placed in a series of 50ml flasks, and hydrochloric acid added to each in different amounts. The volumes of the solutions were increased to approximately 25ml. and the pHs determined. Small adjustments of the pH were made by adding additional sodium hydroxide or hydrochloric acid, in order to give a wide range of pH values. The volumes were

then increased to 30ml with distilled water and final measurements of the initial pH made. 120mg paraformaldehyde (BDH) were added to each flask, giving a 0.13M solution.

The flasks were placed on a sandbath, and the contents allowed to reflux for 5 hours. Approximately 10 minutes was taken for the solutions to boil. The paraformaldehyde dissolved most quickly in those flasks which had extremes of pH, remaining in other cases for up to one hour. After the 5 hours had elapsed, the flasks were removed from the sandbath and chilled. They were then left at room temperature for half an hour before measurements of the final pHs were taken.

A 0.50ml. portion of the solution was diluted tenfold and used for measurement of the absorption maximum in the region 225 - 275nm. (A Pye Unicam Ultra-violet Spectrophotometer SP 800 was employed with 10 or 5mm. quartz cells.) A 0.005ml. portion of this solution was added to the chromotropic acid reagent for formaldehyde determination.

II NaOH - HCl at pH 9.5

A series of flasks containing sodium hydroxide at pH 9.5 (prepared as in I) and 120mg paraformaldehyde was heated at reflux temperature for periods up to 8 hours. At intervals flasks were removed for pH determination.

III NaOH - HCl , 1.20g. paraformaldehyde

As experiment I, but with 1.20g. paraformaldehyde.

IV LiOH - HCl

As experiment I, but with LiOH.

V $Me_4N.OH$ - HCl

As experiment I, but with $Me_4N.OH$

VI $MgCl_2$ - NaOH

10.0ml portions of 0.39M $MgCl_2$ solutions were transferred to a number of flasks and various quantities of sodium hydroxide solution added. After adjusting the volume to 30ml. initial pH measurements were made and 120mg portions of paraformaldehyde were added. The procedure is otherwise as in I.

VII "$AlCl_3$" - NaOH

As in experiment VI, but with "$AlCl_3$". Refluxing periods were as follows:

a. 5 hours,

b. 21 hours, and

c. 100 hours. (The two flasks containing solution at the highest initial pH were not sealed properly and the solvent evaporated.)

VIII $CaCl_2$ - NaOH

As in experiment VI, but with $CaCl_2$.

IX $SrCl_2$ - NaOH

As in experiment VI, but with $SrCl_2$.

X "$TiCl_4$" - NaOH

As in experiment VI, but with "$TiCl_4$".

XI $MnCl_2$ - NaOH

As in experiment VI, but with $MnCl_2$.

XII $FeCl_3$ - NaOH

As in experiment VI, but with $FeCl_3$.

Severe bumping occurred in all the solutions above pH 10.

XIII $CoCl_2$ - NaOH

As in experiment VI, but with $CoCl_2$.

XIV $NiCl_2$ - NaOH

As in experiment VI, but with $NiCl_2$.

XV $CuCl_2$ - NaOH

As in experiment VI, but with $CuCl_2$.

XVI $ZnCl_2$ - NaOH

As in experiment VI, but with $ZnCl_2$.

XVII NaOH - HCl , 2mg. D-glyceraldehyde.

As in experiment I, but with 2mg. D-glyceraldehyde (added in 0.5ml. distilled water).

XVIII NaOH - HCl ,, 21mg NaCN.

As in experiment I, but with 21mg. sodium cyanide (AnalaR, BDH) in 1 ml. distilled water. pH measurements were made only in alkaline solution.

XIX NaOH - HCl , D-glucose - no formaldehyde

As in experiment I, but with 120mg. D-glucose and without the formaldehyde.

CHAPTER 4

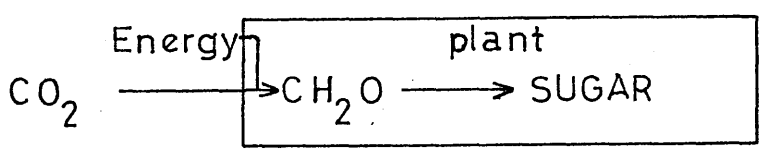
THE FORMALDEHYDE THEORY OF PHOTOSYNTHESIS

Introduction.

So far, we have considered some strictly chemical applications of the formose reaction. The formation of sugars from formaldehyde does however have other fields of relevance and one of these is biological.

Berthelot, in the year before Butlerow's demonstration of the formose reaction, made the hypothesis that sugar formation in plants could involve the intermediacy of formaldehyde[150]

i.e.



This is known as the formaldehyde theory of photosynthesis.

The theory gained considerable support because of the work on the formose reaction and was extended by Baeyer[151] and Loew[152] before the turn of the century. We have already seen that the finding that vitamin C could accelerate the formose reaction was held in great significance. A number of experiments were conducted to show that gaseous formaldehyde could be converted to sugar by plants' leaves:[180] the dried extracts of some plants were even found to be formose reaction promoters.[183]

The first part of the process, the reduction of carbon dioxide in light, is equally important and a considerable number of attempts were made to confirm this part of the theory.
In this chapter we shall review the attempts to reduce carbon dioxide "in vitro". A number of related experiments in which carbon dioxide was reduced to formaldehyde by γ-irradiation, etc., are also included.

TABLE I

ATTEMPTS TO REDUCE CARBON DIOXIDE BY THE USE OF PHYSICAL FORMS OF ENERGY

Abbreviations.

uv.	ultra-violet light	coll.	colloidal
vis.	visible light		
ir.	infra-red light		
sun	sunlight		
γ	gamma rays		
NC	not confirmed		
-	neither formaldehyde nor sugar found		
H	"nascent" hydrogen		
el.	electrical discharge		

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1897	Losanitsch Jovitschitsch	$CO_2 + H_2O$	el.	CH_2O	153
1906	Loeb	$CO_2 + H_2O$	el.	CH_2O	154
1910,	Berthelot,	$CO_2 + H_2O$	uv.	no CH_2O	155,159
1913	Gaudechon	$CO_2 + H_2$	uv.	CH_2O	
		$CO + H_2$	uv.	CH_2O	
1923	Spoehr	repeating 155, 159		NC	171
1911	Stocklasa,	$CO_2 + H_2O$	uv.	-	156
	Zdobnicky	$CO_2 + H_2O + KOH$	uv.	CH_2O	
		$CO_2 + H + KOH$	uv.	sugar	
		$CO_2 + H + KOH$	no uv.	-	

TABLE I cont.

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1912	Stocklasa Zdobnicky	$KHCO_3$ + H	uv.	CH_2O	158
1923	Spoehr	repeating 156, 158		NC	171
1911	Usher, Priestly	$CO_2 + H_2O$	uv.	CH_2O	157
1923	Spoehr	repeating 157	157	NC	171
1913	Moore, Webster	$CO_2 + H_2O$ + coll. $Fe(OH)_3$, uranyl salt	sun	CH_2O	160
1922	Baur, Rebmann	repeating 160		NC	169
1927	Burk	repeating 160		NC	175
1918	Ewart	$CO_2 + H_2O$ + Mg	?	sugar	36,161
1921	Baly Heilbron	$CO_2 + H_2O$ + chlorophyll, methyl orange, malachite green	vis.	CH_2O	163,164
1921	Baly,	$CO_2 + H_2O$	uv.(200nm)	CH_2O	164-7
1924	Heilbron, Barker, Hudson, Stern	$CO_2 + H_2O$ + paraldehyde, NaOPh, metal salts absorbing at 290nm.	uv.(290nm)	CH_2O	
		CO_2 + uranium hydrox., $Fe(OH)_3$	uv.	CH_2O	
		$KHCO_3 + H_2O$	uv.	CH_2O.	
1923	Baur, Buchi	repeating 164		NC	170
1923	Spoehr	repeating 164		NC	171

TABLE I cont.

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1925	Porter Ramsperger	$CO_2 + H_2O$	uv.	-	172
1925	Dhar, Sanyal	$CO_2 + H_2O + FeCl_3$, $CuSO_4$, chromium sulphate, uranyl nitrate, methyl orange, etc.	tropical sun	CH_2O	173
1927	Burk	repeating 173		NC	175
1926	Baly	CO_2 with preactivated coll. catalyst	uv.	CH_2O + sugar	174
1929	Emerson	repeating 173-5		NC	186
1932	Zscheile	repeating Baly		NC	194
1927	Mezzadrolli Gardano	$CO_2 + H_2O$, NH_4HCO_3, $LiHCO_3$, $NaHCO_3$, $KHCO_3$, $Mg(HCO_3)_2$, $Ca(HCO_3)_2$, $Sr(HCO_3)_2$, $Ba(HCO_3)_2$	uv.	CH_2O (sugar)	176
1927	Baly	CO_2 + Al powder, $BaSO_4$, $Al(OH)_3$		CH_2O ?	177
1927	Baly	H_2CO_3 + basic carbonates of Co, Ni	uv.	sugar	179
1928	Mezzadrolli	$Ca(HCO_3)_2$ aq., $KHCO_3$ $KHCO_3$ aq.	uv	CH_2O + sugar	181
		$CO_2 + H_2O$ + Mg, Zn, $Cu(OH)_2$, $Al(OH)_3$, $Ca(HCO_3)_2$, $CaCO_3$, $Ca(OH)_2$	uv.	CH_2O	
1928	Vogel	$CO + H_2$	uv. ?	sugar	182

TABLE I cont.

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1929	Mezzadrolli Giordano	$Ca(HCO_3)_2$	uv.	reducing substance	184
1929	Baly Hood	$NiCO_3$	white light	carbohy.	185
1930-1931	Baly	$NiCO_3$ + aluminated keiselguhr, Fe_2O_3 Cr_2O_3	uv.	CH_2O	187,188
1931	Bell	repeating Baly		NC	190
1931	Dhar, Rao, Ram	$NaHCO_3$ + $NiCO_3$, $Fe(OH)_3$	uv.	CH_2O	189,191
1931	Rao, Dhar	repeating 173 disputing 174		confirmed	191,192
1932	MacKinney	disputing 191			193
		repeating Baly $NiCO_3$, etc.		NC	
1932	Qureschi Mohammad	repeating part of Baly's work		NC	195
1932	Ram, Dhar	CO_2 + H_2O + many coloured catalysts	tropical sun	CH_2O + sugar	196
1932	Ram, Dhar	$KHCO_3$ + Mg + ZnO	sun	CH_2O	197
			no sun	(CH_2O)	
1933	Dhar, Bhargava	many org. compounds	sun	CH_2O (+sugar)	198
1935	Dhar	CO_2 + H_2O + Fe^{III}	light	CH_2O + sugar	199
1935	Baur	CO_2 + H_2O + methylene blue, chlorophyll	sun ?	CH_2O	200

TABLE I cont.

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1960	Mayer, Topelmann	repeating 200		NC	209
1935	Baly	$Al(OH)_3$, Cr_2O_3 Fe_2O_3 + ThO_2 on keiselguhr as catalysts	light	reducing substance	201
1937	Ram, Dhar	corroborating their work	sun	sugars	202
1937	Ostrikov	$CO_2 + H_2O$	uv.	CH_2O	203
		$CO_2 + H_2O + NiCO_3$, $CoCO_3$	uv.	CH_2O	
1942	Dacier	$CO_2 + H_2O$	1400°	cellulose	204
1943	Gardner	doubt cast on role of formaldehyde in plants (Also 180,183)			205
1951	Serono, Marchetti, Gagliardi	$CO_2 + H_2O + Mg$	ir.	CH_2O	206
1951	Calvin, Morrison Hamilton Benson Garrison	$CO_2 + H_2O$	40 Mev. He ions	HCOOH	207,213
		$CO_2 + H_2O + Fe^{II}$	"	CH_2O	

TABLE I cont.

YEAR	INVESTIGATOR(S)	CARBON SOURCE	ENERGY	PRODUCT	REFERENCE
1960	Getoff, Weiss	$CO_2 + H_2O$	γ	CH_2O	208
	Scholes	$CO_2 + H_2O + Fe^{II}$, KI	uv.	CH_2O	
1962	Getoff	$CO_2 + H_2O + Fe^{II}$	uv. $<$ 320 nm.	CH_2O	210
1963	Getoff	$CO_2 + H_2O + Fe^{II}$	uv.	CH_2O	211,212
1970	Horowitz, Hubbard, Hardy	$CO_2 + CO + H_2O +$ soil	uv.	CH_2O ?	214

Discussion.

A considerable number of attempts have been made to establish the 'in vitro' reduction of carbon dioxide. They may be conveniently divided into two sections, chronologically. Those before 1950 were principally concerned with as a part of the formaldehyde theory of photosynthesis: those after this time dealt with the various sources of physical energy that could be successfully employed in the reduction, with a possible relevance of the reaction on other planets of the solar system.

During the earlier period many claims and counter-claims were made, and there was much argument as to whether the reaction occurred at all in the systems that were studied. Table I consists of a summary of the investigators, their systems and their results.

In a short review in 1932 MacKinney[193] summarised part of the work of Baly, Rao and Dhar, who had published many papers in support of the photoreduction, as follows:

"The status of the problem is extraordinarily involved, though it can hardly be doubted that some workers have succeeded in obtaining formaldehyde 'in vitro' .....

One concludes that no procedure has yet been published whereby conditions for obtaining formaldehyde and carbohydrates 'in vitro' can be duplicated in other laboratories."

Dhar continued to publish work after this statement, in support of his earlier findings.

Both gaseous carbon dioxide and dissolved carbon dioxide (carbonate and bicarbonate) have been used as carbon sources. Often a "photosensitiser" has been added e.g. chlorophyll, methyl orange, ferric hydroxide, to aid the reduction. The danger of adding organic compounds to a flask that is going to be subjected to high energy radiation is apparent.

It is important that the apparatus be clean and the chemicals free from contamination.

"Positive tests for formaldehyde were invariably obtained whenever CO_2 came in contact with wax or gease, whereas negative tests were obtained when the joints were replaced by quartz-to-glass seals."[195]

"Merck's chemicals are generally dispatched in glass containers having a coating of wax on the corks or stoppers. Special care has to be exercised in the opening of these containers.... ... positive tests for formaldehyde were often obtained by the use of Merck's extra pure chemical, not a trace of formaldehyde was to be found when sodium bicarbonate prepared in the laboratory with all necessary precautions, was employed."[195]

"Reference to the work of those investigators who have used chlorophyll as a "sensitizer" in their experiments on photosynthesis shows that in most cases little or no mention is made of the state of purity of the preparation. By the word "chlorophyll" one can mean anything from a mixture of green plant pigments to a definite chemical compound known as chlorophyll.[195]

The intensity of the incident radiation may also have an effect.

There is an obvious necessity for carrying out numerous controls and taking all precautions when attempting this kind of experiment. It is felt that great care should be exercised when arguments using as their sole basis these earlier claims, are encountered.

Much of the impetus was taken away from research in this field when the D-ribulose-1,5-diphosphate theory was proposed, after World War II.[149]

The second era bagan with Calvin's demonstration of the reduction of carbon dioxide in the presence of water to formic acid and formaldehyde.[207] Nine years later Getoff and co-workers showed that ^{60}Co γ-rays and ultraviolet light could also be used. The presence of ferrous iron was necessary in the water before the photoreduction could take place.[208-213] Recently Horowitz has isolated organic material, tentatively assigned as formaldehyde, acetaldehyde and glycolic acid, by ultra-violet irradiation of mixtures of carbon dioxide, carbon monoxide, water and soil. It is possible that this type of reaction could occur on Mars, which has a predominantly carbon dioxide atmosphere.[214]

CHAPTER 5

THE FORMOSE REACTION ON THE PRIMITIVE EARTH?

Introduction.

We will consider the possible occurrence of the formose reaction on the primitive earth. The nature of the atmospheres on the earth and other planets of the solar system will be examined, and the "in vitro" demonstration of the formation of formaldehyde from the components of these atmospheres reviewed. As the formation of formaldehyde is endothermic under those conditions the sources of energy available on the primitive earth will be discussed, and their application to the local production of organic compounds considered.

A number of factors that have to be considered in assessing the liklihood of the formaldehyde-sugar conversion on the young planet will be studied in an experimental context.

Discussion.

The formaldehyde theory of photosynthesis does not seem to be applicable to plants, contrary to expectations in the first half of this century (Chapter 4). It may, however, have had relevance elsewhere - on the primitive earth. Here we can see a new connection, a connection with the events on this planet before the dawn of life.

The problem of the origin of life has faced Man for hundreds of years, and many theories have been proposed. Only recently has it been possible to approach the problem scientifically partly because of the theory of chemical evolution.

THE STAGES IN THE EVOLUTION OF THE CELL
(according to Oparin, Bernal, Calvin et al.[217])

SIMPLEST COMPOUNDS OF H, C, O, N

SYNTHESIS (U.V.) OF MONOMERS e.g. amino acids

POLYMERISATION TO MACROMOLECULES
WITH NATURAL SELECTION — e.g. protein, nucleic acid

COLLOIDAL COACERVATES

FORMATION OF CELL MEMBRANE

ANAEROBIC METABOLISM THROUGH FERMENTATION

ADVANCED FORMS OF FERMENTATION INVOLVING HIGH
CONTROL AND ORGANISATION

START OF PHOTOSYNTHESIS

ca. "2.7 x 10^9 years ago
Release of O_2

ESTABLISHMENT OF PHOTOSYNTHESIS

RESPIRATION

INCREASING LEVEL OF
ORGANISATION

The Theory of Chemical Evolution.

This theory commands the largest measure of support at the present time. In it a series of stages in the evolution of the chemical world to the biological have been proposed. They are given on the facing page.

The theory lends itself to two kinds of empirical approach: one begins with the living world and by a series of simplifications attempts to traverse into the non-living; the other moves in the opposite direction. It is with the latter approach that we shall be mainly concerned in this chapter.

Protein, nucleic acid, sugar and fat are present in most organisms. These substances are all based on carbon, hydrogen and oxygen: some contain nitrogen. Chemical evolution requires that these compounds were present on the primitive earth, for organisms to have originated. (This assumes that the principle of biochemical similarity was valid.*)

The atmosphere is generally thought to have been the source of the major bioelements as the crustal abundances of these elements are low. It is then supposed that the primitive gases were subjected to suitable forms of energy in order to make the monomers of protein, sugar, etc.. Once this had been accomplished the formation of complex protein-nucleic acid systems would have the occurred, eventually enabling the creation of a living organism.

What standing does the formose reaction have in this application of the theory?

*The principle of biochemical similarity states that the biochemical constitutions of the first and present life-forms are the same e.g. the genetic material has always been nucleic acid.
A different approach is adopted in Chapter 6.

The Formose Reaction and the Theory of Chemical Evolution.

Most of the chemical evolutionary experiments have been directed towards the formation and study of systems involving protein and nucleic acid. The formose reaction has received scant treatment.

Gabel and Ponnamperuma demonstrated the formation of sugars from formaldehyde in the presence of alumina, kaolinite and illite:[119] and Reid and Orgel used a carbonate-apatite mixture for the same purpose, although they were unable to find the phosphorylated sugars that they sought.[120] Ribose and 2-deoxyribose have been found in the formose caused by ultra-violet light.[113] All these experiments have been done in the last six years.

In a few books and reviews on the origin of life the number of pages devoted to the formose reaction is approximately ½ in 39,[218] 2 in 278,[219] 2 in 301,[220] 3 in 193,[221] and 3 in 482.[222] Discussion is confined to an acknowledgment that sugars can be formed from formaldehyde (lime and Butlerow are often mentioned), and to a diagram inter-relating the sugars (usually like Pfeil and Rucket's - Chapter 1), and to the finding that ribose and 2-deoxyribose can be present in formose.

Apart from showing that sugars are not too difficult to make, the main significance attached to the formose reaction is in its ability to form (sometimes) the nucleic acid sugars.

The importance attached to protein and nucleic acid lies in their involvement in enzymes and the genetic material, respectively. They contain specific information. Fats and sugars, on the other hand, are more important as structural units and energy stores. Chemical evolutionists therefore feel that it is more profitable to study the information-containing systems, if an origin of life is to be found.

In order to find whether the formose reaction was likely on the primitive earth it must first be established that formaldehyde can be formed from the components of the early atmosphere.

TABLE A.

PROBABLE ATMOSPHERES OF THE EARTH

(after Cloud,[223]* and Holland.[224]**)

	TIME AGO IN AEONS (i.e. 10^9 years)			
	4.5*	4.5 - 4.0**	4.0 - 2.0**	2.0 - present*
MAJOR CONSTITUENTS	inert gases	CH_4 H_2 (?)	N_2	N_2 O_2
MINOR CONSTITUENTS		H_2 (?) H_2O N_2 H_2S NH_3 Ar	H_2O CO_2 Ar	Ar H_2O CO_2
TRACE CONSTITUENTS		He	Ne He CH_4 NH_3 (?) SO_2 (?) H_2S (?)	Ne He CH_4 Kr

TABLE B.

CONSTITUTION OF THE PRIMITIVE ATMOSPHERE [246]

a.	CH_4, NH_3, H_2O, H_2	Oparin, Urey
b.	$CH_4 \longrightarrow CO_2$: $NH_3 \longrightarrow N_2$: H_2O: H_2	Bernal
	CO_2, NH_3, H_2S, H_2O	
c.	CO_2, N_2, H_2S, H_2O	Rubey
	CO_2, NH_3, H_2S, H_2O	
d.	CO, CO_2, N_2, H_2S, H_2O	Revelle
e.	$CH_4 \longrightarrow CO \longrightarrow CO_2$	Dauvillier

TABLE C.

ATMOSPHERES OF THE INNER PLANETS[240]

		CO_2	N_2	H_2O	O_2
VENUS	atmosphere	70,000	<3,000	~10	<10
	crust	-	-	-	8×10^6
	oceans	-	-	-	-
EARTH	atmosphere	~11	800	~1	200
	crust	70,000	2,000	-	8×10^6
	oceans	-	-	300,000	-
MARS	atmosphere	70	< 1	< 0.01	≈0.1
	crust	-	-	-	-
	oceans	-	-	-	-

Units - $g.cm.^{-2}$

The Atmospheres of the Earth and Other Planets in the Solar System.

Our atmosphere is of comparatively recent origin and is distict from the atmospheres on other planets of the solar system. This is because life is present on the earth: plant photosynthesis produces oxygen in large quantities, a gas which does not exist on any other planet in a significant amount.[235] The precise history of the earth's atmosphere has not been finally established, but the main trends are becoming clearer.

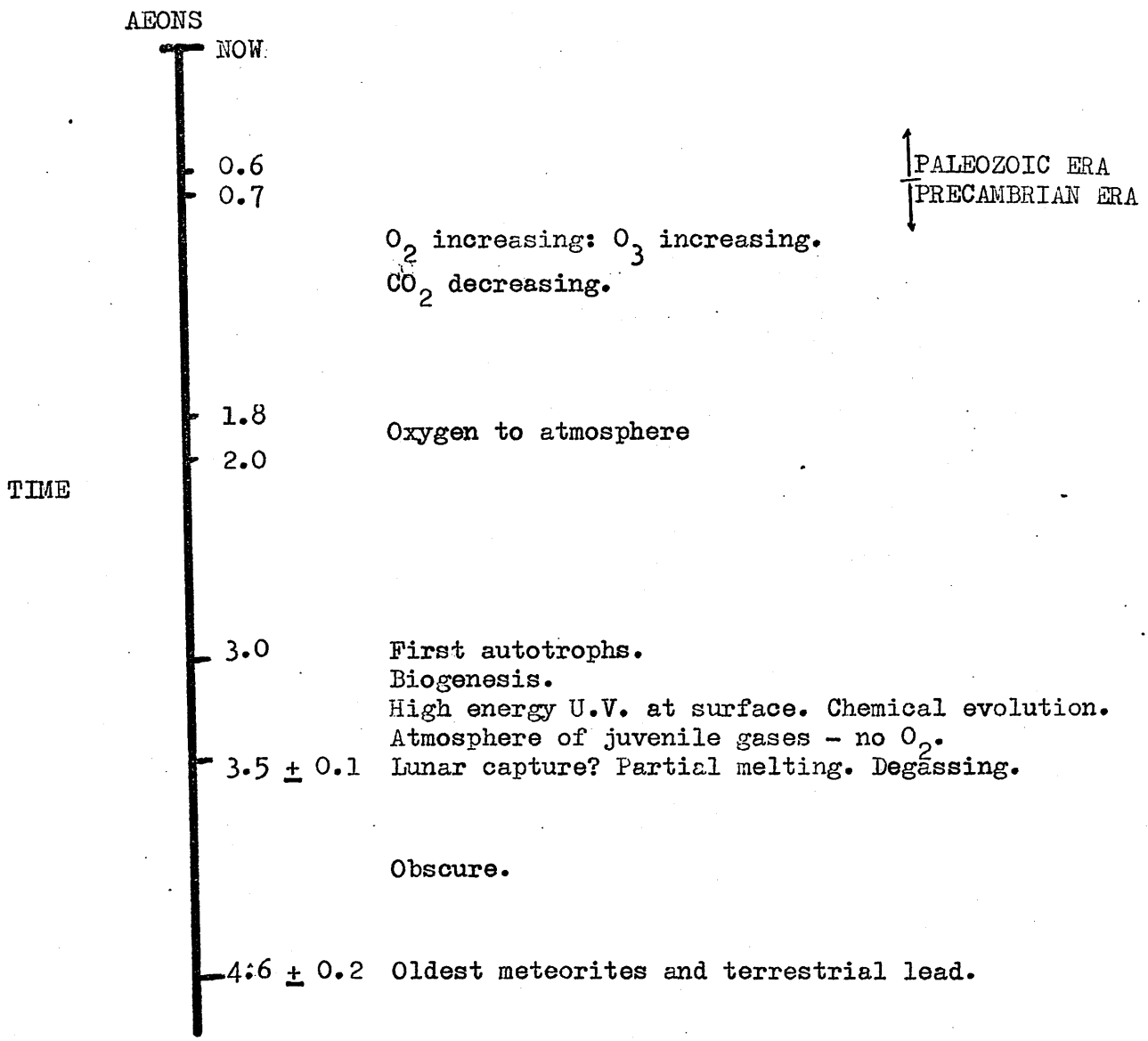
Inert gases were most probably the major components of the first atmosphere, resulting out of the high levels of radioactivity on the juvenile planet.[227] This atmosphere was somehow lost, being replaced by one containing carbon, hydrogen, nitrogen and oxygen. Two types of this kind of atmosphere can be identified. One contains molecular hydrogen: the carbon, nitrogen and oxygen are then in a reduced form viz. CH_4, NH_3, H_2O. The other lacks molecular hydrogen and the resulting atmosphere is more oxidised, CO_2, N_2, H_2O. The former is basic (because of the ammonia): the latter is acidic (because of the carbon dioxide). The stages in the development of the atmosphere have been discussed elsewhere.[227,228,232,233,237,238,239] (Tables A,B)

The favoured atmosphere for chemical evolution experiments is the reduced one, probably because the evidence for the carbon dioxide-type of atmosphere is comparatively recent. Carbon dioxide and its accompanying atmospheric components may have resulted from volcanic outgassing.[225,228,230,231,233]

The atmospheres of other planets in the solar system fall into two categories. The inner planets, Mars and Venus, have predominantly carbon dioxide atmospheres (Table C). Mercury either has no atmosphere, or possibly one containing a very little CO_2.[235] The outer (Jovian) planets have methane based atmospheres.[236] We might expect that the earth

TABLE D.

THE MAIN FEATURES OF THE EVOLUTIONARY INTERACTION OF THE EARTH'S BIOSPHERE, LITHOSPHERE AND ATMOSPHERE (HYPOTHETICAL)*



* After Cloud.[233]

TABLE E.

ENERGY SOURCES IN THE TERRESTRIAL ENVIRONMENT

ENERGY SOURCE	ENERGY AVAILABLE $cal.cm.^{-2}yr.^{-1}$	ENERGY AVAILABLE 4×10^9 YEARS AGO $cal.cm.^{-2}yr.^{-1}$
Solar Radiation		
total	260,000	< 260,000
3500Å	9,000	< 9,000
2500Å	400	< 400
2000Å	50	< 50
1500Å	2	< 2
Geothermal Energy	50	> 50
Electrical Discharges	4	< 4
Volcanoes	0.13	?
Radioactivity (outer 10 m.)	0.008	0.028
Cosmic rays	0.0015	?
Sonic Energy in Sea	0.0001	0.0001
Meteorites	0.00004	?

would have had an atmosphere similar to that of Mars or Venus, had not life been present.

The time at which life arose on the earth is of course important, for then we can find the type of the contemporary atmosphere. One estimate of the time of the origin of life is given in Table D, 3.0 - 3.5 aeons ago. Comparison of this figure with Table A places the origin in a carbon dioxide era. We should remember that there is a measure of uncertainty attached to both tables.

In the following arguments are arguments are applied principally to the primitive earth: they may be applicable to other planets.

Sources of Energy on the Primitive Earth

The formation of the high energy monomers from the atmospheric gases is endothermic. Life today ultimately depends on the sun's luminous energy, but other energy sources may have been important in the formation of organic compounds from the atmosphere of the young earth.

The energy sources that might have been available on the earth have been quantified: the list was recently revised by Hulett.[241] We can add yet another energy source, geothermal energy, which is due to the cooling of the earth's interior. Mason[242] has estimated that the present value of this is 50 50 $cal.cm.^{2}yr.^{-1}$, and it would be expected that it would be larger 4 aeons ago with the more intense levels of radioactivity. Geothermal energy is therefore the second largest contributor of energy (Table E).

TABLE F.

THE FORMATION OF FORMALDEHYDE FROM MIXTURES OF ATMOSPHERIC GASES

GASEOUS MIXTURE	ENERGY SOURCE	PRODUCT	REFERENCE
$CO_2 + H_2$	uv.	CH_2O	244
	e^-.	CH_2O	245
$CO + H_2$	corona tube	$C_n(H_2O)_n$	244
	electrical discharge	sugar	244
	electrical discharge	CH_2O	244
	ultrasonics	CH_2O	247
$CO + H_2 + N_2$	ultrasonics	CH_2O	247
($CH_3OH + H_2O + N_2$	electrical discharge	CH_2O	245)
$CH_4 + CO_2$	e^-.	CH_2O	245
$CH_4 + CO$	uv.	CH_2O	244
$CH_4 + H_2O$	e^-.	CH_2O	245
$CH_4 + NH_3 + H_2O$	uv.	CH_2O	247,249
(CO_2 reduction	See Chapter 4)		

Abbreviations: uv. ultra-violet light

e^-. electron irradiation

The Formation of Formaldehyde from Mixtures of Atmospheric Gases.

Suppose that we have an atmosphere of carbon, hydrogen and oxygen in some form. Can formaldehyde be formed?

In Chapter 4 we reviewed the attempts to reduce formaldehyde "in vitro", and although there was some doubt attached to the earlier work, the more recent work strongly points to the formation formaldehyde. Table F describes some of the gaseous mixtures that are said to produce formaldehyde after their subjection to high energy irradiation. We can see from this list that a number of mixtures have been used, and that differing energy sources can have the same net effect.

We may conclude that, if this work is reliable, formaldehyde would not be too difficult to make in some of the primitive atmospheres.

The Question of Concentration.

Evidence for formaldehyde's synthesis as above is a necessary condition for the formose reaction, but not a sufficient one for, if the formose reaction is to occur, a sufficiently high concentration of formaldehyde must be present at or near the earth's surface.

The notion of the oceans of the primaeval world containing "chemical" levels of concentration is commonly encountered in chemical evolution theory, being frequently described as the "primitive soup". In order to achieve these levels of concentration in the oceans, a vast amount of energy would have had to be expended.

Hulett has calculated that the equilibrium concentration of formaldehyde in the oceans would have been 10^{-9}M, and that the rate of formaldehyde production from a CH_4/CO mixture under the influence of solar energy would have been 10^{-64} mol.cm.$^{-2}$yr.$^{-1}$.[241] We would expect other figures from other gaseous mixtures, but it would seem that very low levels of concentration would be achieved through general production.

Reid and Orgel showed that the minimum formaldehyde concentration for the formose reaction was approximately 10^{-2} to 10^{-3}M in the presence of calcium carbonate.[120] Ponnamperuma, however, succeeded in producing sugar under ultra-violet rays from 3 x 10^{-4} formaldehyde solution.[113] These concentrations are fairly high (c.f. 10^{-9}M.) so if the formose reaction is to be found on the primitive earth locally high concentrations will be required. Let us examine the general requirements for local production of organic compounds.

TABLE G.

ENERGY SOURCES AND THEIR ZONES OF EFFECT

ENERGY SOURCE	ATMOSPHERE		HYDROSPHERE			LITHOSPHERE	
	UPPER	LOWER	DEPTHS	SURFACE	SHORE	SURFACE	CRUST
Solar Radiation	+	+		+	+	+	
Geothermal Energy	?	?	+	+	+	+	+
Electrical Discharges	+	+		?	?	?	
Volcanoes		+			?	+	+
Radioactivity		?			?	+	+
Cosmic Rays	+	+	+	+	+	+	+
Sonic Energy in the Sea		?	?	+	+		
Meteorites	+	+		?	?	?	

(The oceanic bed has been omitted from the above list for reasons of space. Geothermal, radioactive and volcanic energies may affect this zone.)

Local Production: General Requirements.

For local production to occur, the energy, the material or specific catalysts for the reaction must be available on the surface of the earth, in higher-than-average quantity. The supply of the product will be limited by the least available of the presursors. (Here we are assuming that no side reactions need be considered.)

a. Local Energy Sources.

If we examine the table of energy sources given previously, we can see that solar energy's contribution is greater over-all than the others - indeed solar energy is the energy source on which life on the earth ultimately depends. It would be misleading to assume, however, that solar energy was necessarily the most important factor in the formation of organic compounds on the primitive earth. Averages are misleading, for in local environments other forms of energy may be more important.

Each energy form has a different zone of action. Solar radiation will be most important in the atmosphere and on the surface of the earth (land and sea). Radioactivity will be most important under the surface of the earth. Each of the energy sources has been classified for its zone(s) of greatest effect (Table G). The zones are self-explanatory and no definitions will be given. Also, a measure of subjective assessment applies as to whether solar energy, say, is important in the oceanic depths.

Each energy will have a different way of expending its energy: we might call this the "wattage". Solar radiation is constant during the day, but is non-existent at night. Volcanoes emit a large amount of energy in a short time: their zone of application is limited, that of solar energy is widespread. Electrical discharges have high local energy expenditure and are not confined to the same places, like volcanoes (Table H).

TABLE H.

CHARACTERISTICS OF EACH ENERGY TYPE

ENERGY SOURCE	CHARACTERISTICS	
	ENERGY SPREAD	PERIODICITY AND LOCALE
Solar Radiation	General distribution:	diurnal occurrence
Geothermal Energy	General distribution:	continuous occurrence
Electrical Discharges	Localised distribution:	aperiodic, not in the same place
Volcanoes	Localised distribution:	aperiodic, in the same place
Radioactivity	Localised distribution:	continuous, in the same place
Cosmic Rays	General distribution:	aperiodic, not in the same place
Sonic Energy in the Sea	General distribution:	constant(?),in the same place
Meteorites	Localised distribution:	aperiodic, not in the same place

Table H describes the type of expenditure that we can ascribe to each source.

The net result of energy expenditure depends on the balance between its formative and destructive effects.

b. Local Material Sources.

Local production of organic compounds is still possible if the atmospheric gases can be concentrated in one place. All of the gases may be removed from the atmosphere given a sufficiently low temperature, but in practice ammonia, carbon dioxide and water would be the most susceptible to this form of physical leaching. Chemical leaching may also occur, such as the dissolution of carbon dioxide and ammonia in water.

c. Local Catalyst Sources.

A catalyst for a particular reaction may be present in a local environment where neither energy nor material is especially concentrated.

(We shall not consider physical methods of concentrating organic material e.g. concentration by evaporation of water in a pool which has a wide catchment area, although there is no reason why such phenomena may not have occurred. Here general production of material could be a satisfactory means of providing the various compounds.

Chemical evolution theory uses this type of principle to provide the primitive, but oceanic,"soup". Smaller volumes of water would concentrate the components much quicker,provided that evaporation could occur.)

Fig. I

ULTRA - VIOLET ABSORPTION SPECTRUM OF CARBON DIOXIDE [250]

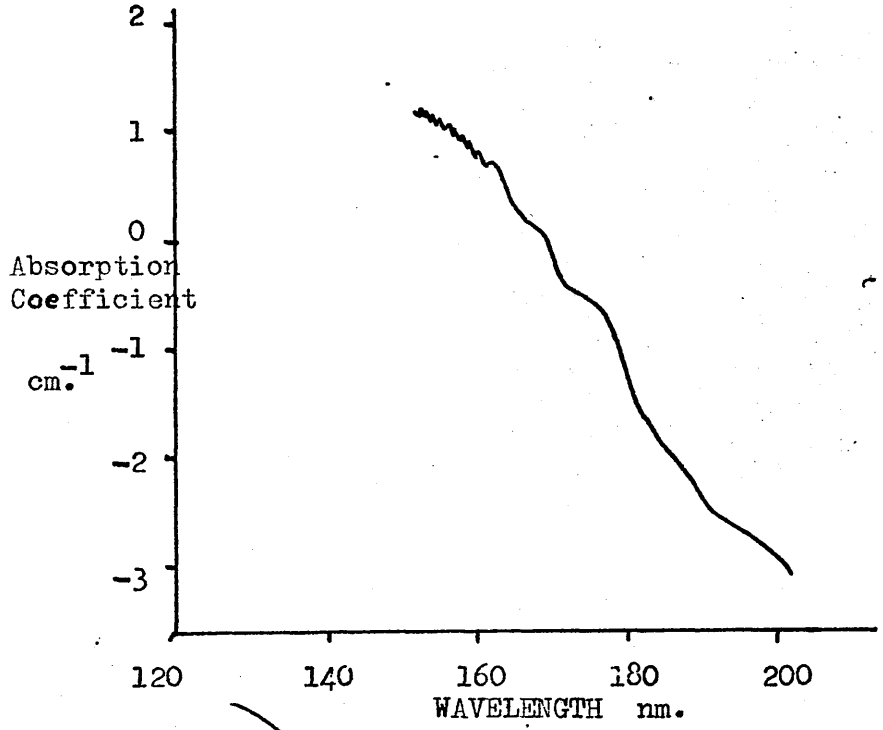
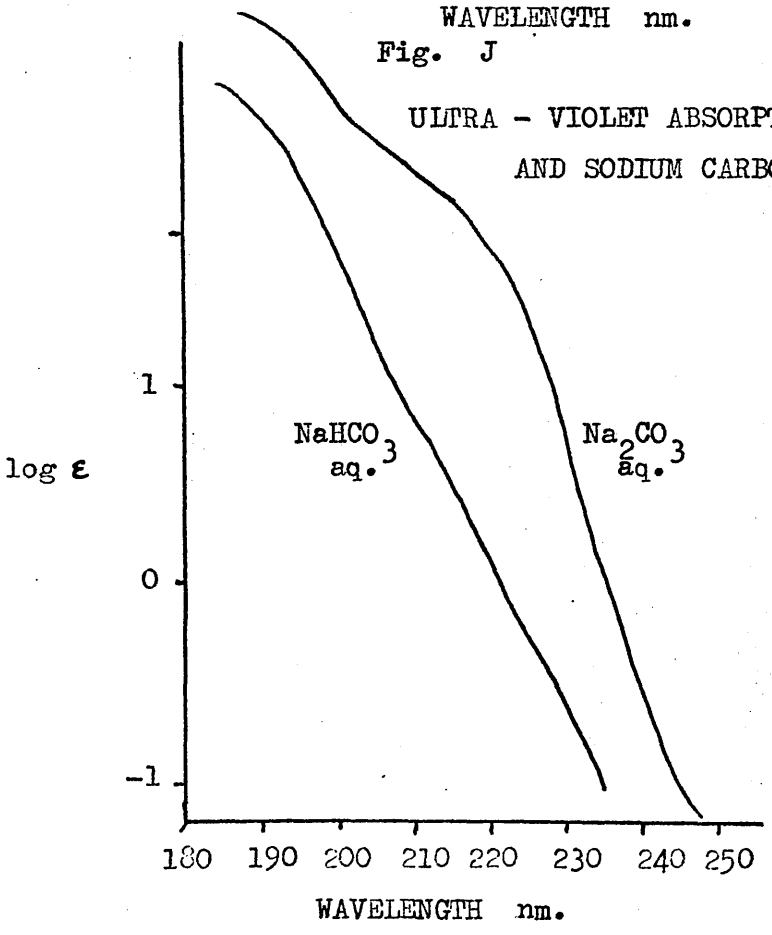


Fig. J

ULTRA - VIOLET ABSORPTION SPECTRA OF SODIUM BICARBONATE AND SODIUM CARBONATE (AQUEOUS) [251]



A Hypothetical Method for Local Production of Formaldehyde.

We have looked at the general requirements for local production. Let us now take a specific example - how to attain locally high concentrations of formaldehyde.

For this we shall choose a carbon dioxide-containing atmosphere, like Mars and Venus have and perhaps the primitive earth had. We shall use the solar ultra-violet light as our energy source, and shall assume that water is present.

Solar radiation is distributed generally over the surface of the earth. Carbon dioxide will absorb part of this radiation and allow the remainder to pass to the surface of the earth. (The presence of water in the atmosphere will probably cause the formation of formaldehyde in the upper atmosphere but we shall not be concerned with that.) Some carbon dioxide will dissolve in the water on the surface of the earth to form carbonate and bicarbonate ions. The ultra-violet absorption spectra of carbon dioxide, sodium carbonate and sodium bicarbonate are recorded in Figs. I and J. From these we can see that the longer wavelengths of the ultra-violet radiation, which are not absorbed by carbon dioxide, can be absorbed by aqueous carbonate and bicarbonate ions.

This longer-wavelength radiation may cause the formation of CH_2O in the presence of Fe^{II} (Getoff has shown that this aldehyde is formed by ultra-violet irradiation of carbon dioxide, in the presence of an aqueous solution of ferrous sulphate. See Chapter 4.)

The formaldehyde that is then formed may hydrate to some extent, forming polyoxymethylene polymers if the concentration of formaldehyde is high. Both of these remove a large proportion of the formaldehyde from the influence of the longer-wave radiations that reach the surface of the earth: they will lie under the carbon dioxide screen. (The ultra-violet absorption

Fig. K

ULTRA - VIOLET ABSORPTION SPECTRUM OF WATER [252]

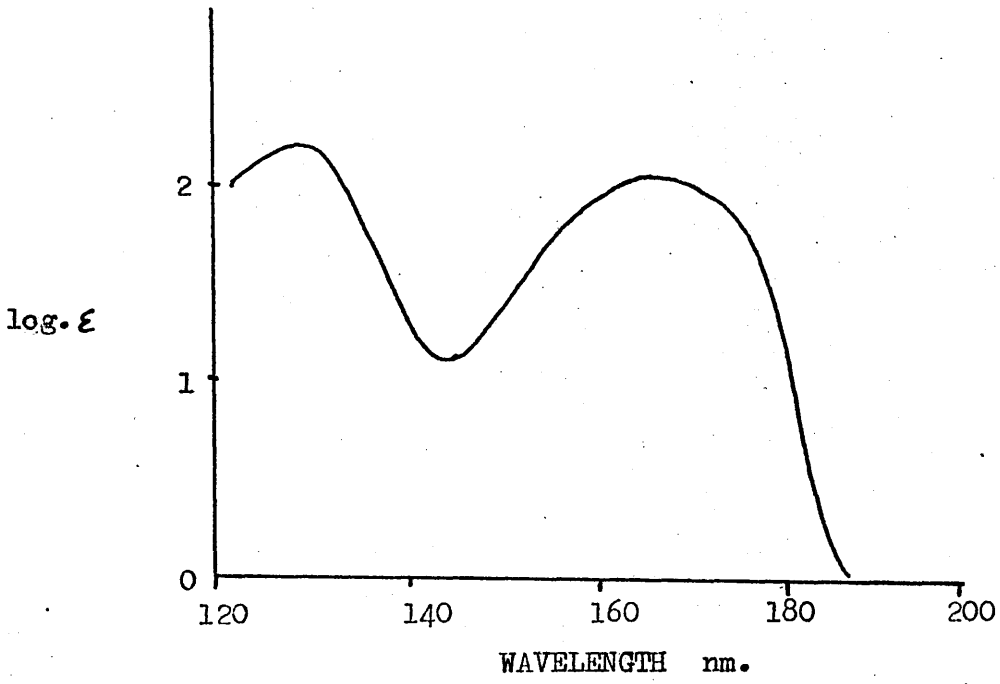
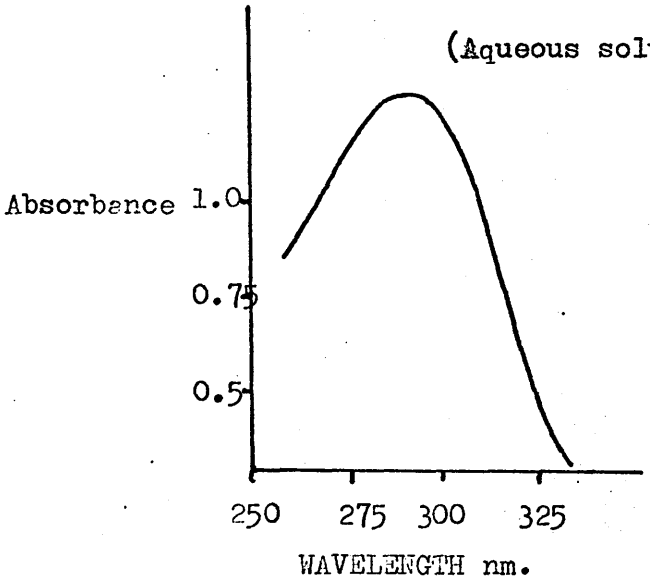


Fig. L

ULTRA - VIOLET ABSORPTION SPECTRUM OF FORMALDEHYDE [253]



spectra of formaldehyde and water are given in Figs. K,L. We can see that water itself would not seriously affect the previous argument.)

The possibility of local concentrations of formaldehyde was discussed by Horowitz, who suggested that dust might help to remove much of the formaldehyde from the harmful ultra-violet light, by burying it.[214] It was also suggested at one time that the white clouds on Venus might have been due to polyoxymethylene aerosols.[254]

TABLE I

PRINCIPAL CONSTITUENTS OF THE ROCKS

All the rocks come from Scotland (including "unknown"s) except where stated.

SILICIC ACID*	Mallinckrodt Chem. Works, USA.	$Si(OH)_4$
BIOTITE	Morven	K, Mg, Fe^{III}, Al fluoro-silicate
GALENA	Lead Hills	PbS
QUARTZ I	Unknown	SiO_2
QUARTZ, MICA SCHISTI	Morven	SiO_2; K, Al silicate
ANGLESITE I	Lead Hills	$PbSO_4$
QUARTZ, BIOTITE I	Morven	SiO_2: K, Mg, Fe^{III}, Al. flourosilicate
HORNEBLENDE	Ardnamurchan	Na, Mg, Ca, Fe^{III}; Al flourosilicate
SERPENTINE	Cornwall, England	Mg silicate
QUARTZ, BIOTITE II	Ballachullish	see above
SILICA*(acid washed)		SiO_2
SLATE	I. of Rum	Al silicate
ZINC BLENDE	Tyndrum	ZnS
GYPSUM	Strathblane	$CaSO_4$
CHALCOPYRITES I	Strontian	$CuFeS_2$
ALUMINA (neutral)*	M.Woelm, Eschwege, Germany	Al_2O_3
ANGLESITE II	Lead Hills	$PbSO_4$
QUARTZ, MICA SCHIST II	Morven	see above
QUARTZ II	Unknown	SiO_2
QUARTZ III	Unknown	SiO_2

TABLE I cont.

ALBITE	Morven	Na, Al silicate
GRANITE	Jersey, Channel Is.	K, Al silicate
QUARTZ IV	Unknown	SiO_2
CALCITE I	Unknown	$CaCO_3$
CHALCOPYRITES II	Strontian	$CuFeS_2$
PREHNITE, ANALCITE, NATROLITE	Barrhead	Na, Ca, Al silicate
CALCITE III	Unknown	$CaCO_3$
CALCITE IV	Barrhead	$CaCO_3$
CALCITE V	Unknown	$CaCO_3$
CALCITE iceland spar VI	Unknown	$CaCO_3$
GALENA, ANGLESITE, CERUSSITE	Lead Hills	PbS, $PbSO_4$, $PbCO_3$
LIME*	BDH	CaO

* Laboratory reagent.

THE FORMOSE REACTION AS A POSSIBLE PREBIOLOGICAL OCCURRENCE

Suppose, then, that formaldehyde is available on the primitive earth. How widespread will the formose reaction be, and what sorts of thing will affect it?

The Generality of the Formose Reaction.

Some naturally occurring minerals are known to catalyse the formose reaction - lime, chalk, alumina, kaolinite, illite, and calcites (Chapter 1). I have extended this list by taking samples of different kinds of rocks that are found on the present-day earth, and examining their abilities to convert formaldehyde to sugar. Approximately thirty rocks were selected, and their major constituents identified (Table I). These rocks were not pure in the chemical sense. It was not intended that they should be. They were mixtures of minerals, some in trace amounts. By using them as possible formose catalysts we should be able to say with a greater degree of certainty whether the formose reaction could have occurred on the primitive earth.

Formaldehyde was added to the powdered rocks and then refluxed in their presence for five hours under nitrogen. The products were then analysed for sugar. At the end of the reflux time some of the solutions were seen to be brown, and they possessed the odour of caramel. A formalised chromatogram of the products is given in Table I'. (This was obtained by the use of the aniline oxalate spray.)

The rocks have been arranged in order of the pH which they exerted in water. Final pHs were also recorded. It is obvious that the hydroxide ion was not the sole catalyst for the reaction, although it did have an effect. The products behaved chromatographically as C_2 to C_6 sugars. Their colour reactions to various reagents were those that would be expected for the same sugars. The colour and odour of the solutions

TABLE I

ROCK'S MAIN CONSTITUENT	INITIAL pH	FINAL pH	FINAL CH_2O
SILICIC ACID	4	5	+
BIOTITE	5½	4½	+
GALENA	6	6	-
QUARTZ I	6	6	+
QUARTZ, MICA SCHIST I	6½	5	+
ANGLESITE I	6½	6½	+
QUARTZ, BIOTITE I	6½	5½	+
HORNBLENDE	7	5	(+)
SERPENTINE	7	7½	+
QUARTZ, BIOTITE II	7	7	+
SILICA (ACID WASHED)	7	5	+
SLATE	7	6	+
ZINC BLENDE	7	7	+
GYPSUM	7½	6½	+
CHALCOPYRITES I	7½	6	+
ALUMINA (NEUTRAL)	7½	6	-
ANGLESITE II	7½	7	(+)
QUARTZ, MICA SCHIST II	7½	7½	(+)

FORMALISED CHROMATOGRAM

SOLVENT FLOW

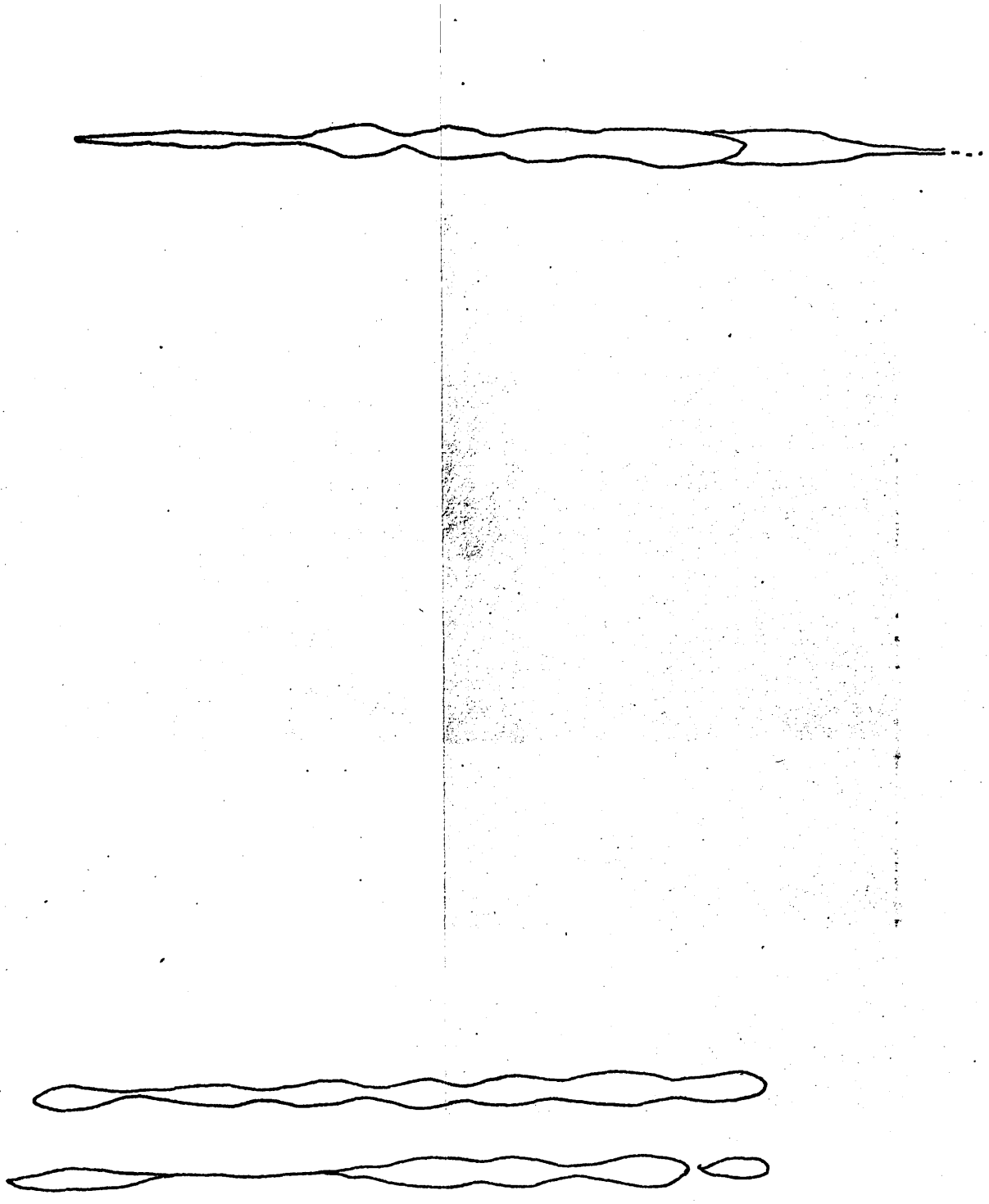
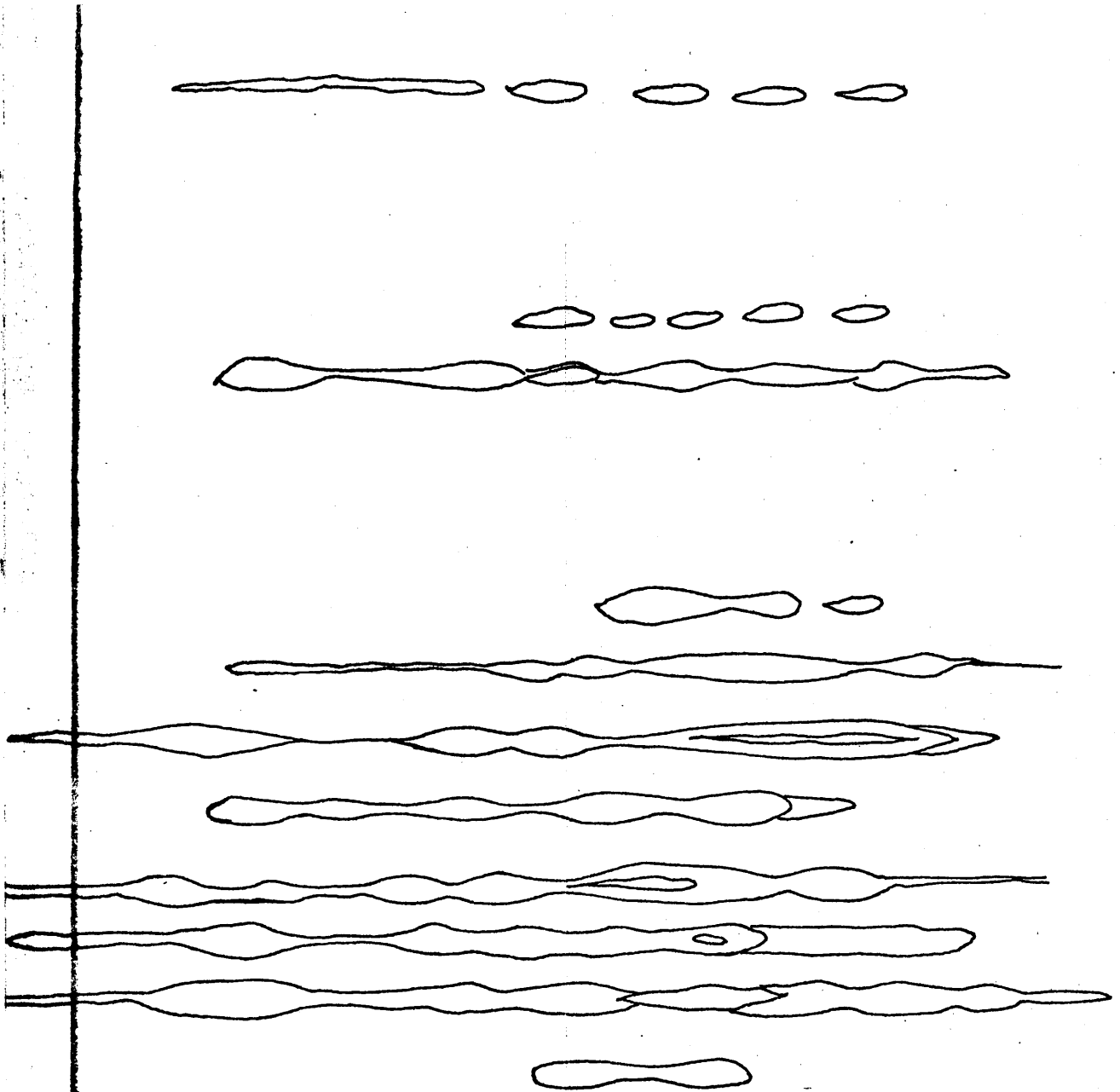


TABLE I' cont.

ROCK'S MAIN CONSTITUENT	INITIAL pH	FINAL pH	FINAL CH_2O
QUARTZ II	7½	4½	+
QUARTZ III	7½	7½	(+)
ALBITE	8	7	+
GRANITE	8	6½	+
QUARTZ IV	8	7½	(+)
CALCITE I	8	7½	(+
CHALCOPYRITES II	8	8	+
PREHNITE, ANALCITE, NATROLITE	8	8	+
CALCITE II	8½	8	(+)
CALCITE III	8½	8	+
CALCITE IV	8½	7½	-
ALUMINA (NEUTRAL: HEATED)	9½	6	-
CALCITE V	9	6½	-
CALCITE VI (ICELAND SPAR)	9	7	-
GALENA, ANGLESITE, CERUSSITE	9	6½	-
LIME	13½	13½	-

FORMALISED CHROMATOGRAM

SOLVENT FLOW



were of caramel. It is highly probable that sugars had been formed.

The distributions of the sugars varied from one rock to another. The products from neutral alumina were chromatographed many times, and each time the distribution of sugars was the same. We may accept that the distribution for each catalyst is constant, and perhaps characteristic. Sugars containing more than six or seven carbon atoms were not detected although there was some evidence of streaking. It is thought that the streaking was due to a salt effect, rather than oligosaccharide formation; no discrete spots were observed at $R_{glucose}$ values less than hexose.

Formaldehyde was estimated qualitatively at the end of the reaction and it was found that in the majority of cases where sugars had been formed no formaldehyde remained (in accordance with expectation).

The pH values of nominally the same mineral in Table I' may differ. This is due to the presence of the trace minerals which were not identified. In most cases the pH fell. This may have been due either to the Cannizzaro reaction, or to the dissolution of the rock in water (or both). pH increases are probably due also to the dissolution of the rock (see Chapter 2 for the pH changes on the dissolution of alumina in water).

Neither sugar nor formaldehyde was detected after extraction of a number of the rocks with water over five hours (Ib). The effect of hydroxide ion alone on formaldehyde solution was mentioned in Chapter 3 - in the presence of sodium ions we would not expect any sugar below pH 11.5.

Often caramel was formed. Usually this was not severe, if the colour of the solution can be used as a guide. Only lime caused severe caramelisation. A white spot of unknown

TABLE I"

OXIDES, HYDROXIDES, AND CARBONATES AS FORMOSE CATALYSTS

CATALYST	INITIAL pH	FINAL pH	FINAL CH_2O*	COLOUR OF SOLUTION
SnO_2	4.1	4.1	+	clear
TiO_2	6.2	5.8	+	clear
PbS	6.3	6.2	+	clear
$PbCO_3$	6.5	5.5	-	brown
$CuCO_3$	6.5	5.5	+	(green)
$SrCO_3$	7.7	8.9	-	brown
ZnO	8.0	7.0	+	clear
$CaCO_3$	8.1	7.4	-	brown
$CoCO_3$	8.3	8.8	-	(red)
$BaCO_3$	8.4	8.2	-	brown
$ZnCO_3$	8.6	8.9	(+)	brown
CuO	8.7	6.9	+	clear
$NiCO_3$	8.7	8.9	+	(green)
$Al_2(CO_3)_3$	9.4	9.7	-	brown
$CdCO_3$	9.8	7.3	-	brown
MgO	10.2	9.7	-	clear
$Ba(OH)_2$	13.0	13.0	-	brown
$Ca(OH)_2$	13.4	13.3	-	brown

* Chromotropic acid method.

origin was observed after the aniline oxalate-sprayed chromatogram had been allowed to deteriorate for several days (by this time the rest of the chromatogram was yellow). With lime this spot was more marked than with any of the other catalysts: it appeared in the latitudes of tetrose and pentose.

Under the conditions of the reactions, approximately one half of the rocks were formose catalysts. In an attempt to initiate the second stage of the reaction for some of those rocks that did not catalyse sugar formation, small amounts of 1,3-dihydroxyacetone were added to the formaldehyde prior to reflux. With slate, horneblende, silica, albite and granite, some of the previously inactive catalysts no change was observed (Id).

A number of oxides, hydroxides and carbonates, this time not naturally occurring, were examined under the same conditions as before. The formation of sugar, which is indicated by a brown colouration, of the solution was found in approximately half of the cases (Table I"). As in Table I' the list is in order order of pH: again we can see that the pH was not the only factor involved in the catalysis, and that where sugar had been formed no formaldehyde remained.

The results in Tables I' and I" should be treated with a measure of caution - we saw in Chapter 3 that a slight difference in pH could make the difference between "formose" and "no formose".

TABLE II

THE FORMOSE REACTION AT ROOM TEMPERATURE

ROCK	TIME	CTA DETERMINATION OF FORMALDEHYDE	U.V. ABSORBANCE OF SOLUTION
SILICIC ACID* Mallinckrodt Chem. Works, USA	38 months	+	0
GALENA Lead Hills	"	+	0
ANGLESITE Lead Hills	"	+	0
ALUMINA (neutral)* M.Woelm, Eschwege, Germany	"	+	?
IRON PYRITES Unknown	"	+	0
QUARTZ Unknown	"	+	0
CALCITE Unknown	"	+	0
CALCITE Unknown	"	+	0
CALCITE Unknown	"	+	0
CALCITE (Iceland spar) Unknown	"	+	0
GANENA, ANGLESITE, CERUSSITE Lead Hills	"	+	0
LIME* BDH	"	0	+

* Laboratory Reagent.

All reactions were carried out in 0.13M formaldehyde solution.

TABLE III

THE EFFECT OF ALTERING THE AMOUNT OF ALUMINA RELATIVE TO A FIXED AMOUNT OF FORMALDEHYDE

MASS OF ALUMINA IN 15ml. 0.13M AQUEOUS PARA-FORMALDEHYDE SOLUTION	INITIAL pH*	FINAL pH**	CTA DETERMINATION OF CH_2O AT END	FINAL COLOUR OF Al_2O_3
100mg.	7.1	5.7	++	white
500mg.	7.2	5.8	++	white
1g.	7.2	5.5	+	cream
2g.	7.4	6.4	0	v. yellow
5g.	7.9	6.5	0	v. yellow
10g.	8.1	7.4	0	yellow

* Initial pHs were determined after allowing the alumina and formaldehyde to sit for 12 hours at room temperature.

** Final pHs were determined after the formaldehyde solution had been refluxed for 5 hours.

The Formose Reaction at a Lower Temperature.

The prevailing temperature on the primitive earth may not have been as high as 100° although there would have presumably been geothermal springs. A number of rocks were left in contact with formaldehyde solution at room temperature for periods up to 38 months (Table II). Formaldehyde remained in the majority of reactions, but where lime was present none was left. A brown colouration appeared over the surface of the lime after a few weeks. This disappeared on shaking, and never returned to its original intensity. It is most probable that this colour was due to caramel.

Catalysts which were able to catalyse sugar formation at 100°, such as alumina, failed to do so at room temperature. Pfeil[78] has already noted that the formose reaction may not occur under 70° in some systems.

On the basis of results obtained in Chapter 3, it would seem likely that strontium ions would also be able to catalyse the reaction at room temperature or slightly above.

SOME FACTORS AFFECTING THE FORMOSE REACTION ON THE PRIMITIVE EARTH

A number of rocks have been shown to catalyse the formation conversion of formaldehyde to sugars. Let us now look at some of the other factors which have to be considered.

Alumina was used as the catalyst of choice for the experiments. It had the ability to form sugars easily under our conditions without causing excessive destruction. It was also available in quantity.

i. The Amount of Catalyst.

The effect of altering the amount of catalysts relative to a fixed amount of formaldehyde was mentioned in Chapter 2. Table III shows that a fourfold difference in the amount of alumina can determine whether sugar is formed. This may be due partly to the pH and partly to the alumina-formaldehyde ratio. Curves for the consumption of formaldehyde with acid, neutral and basic alumina were given in Chapter 2.

ii. The Concentration of Formaldehyde.

Were the arguments that were given for local production of formaldehyde really necessary?

Gabel and Ponnamperuma isolated only glycolaldehyde after reactions with 0.001M formaldehyde solution.[119] Reid and Orgel were unable to isolate any sugars at a similar concentration and have used this as an objection to the formose reaction on the primitive earth.[120] Ponnamperuma successfully converted formaldehyde to sugars at pH 4.5 by the aid of ultra-violet rays: the formaldehyde concentration here was 0.0003M. Hence in the absence of ultra-violet rays, a relatively concentrated solution of formaldehyde will be necessary before the formose reaction will occur. (We shall not consider the effect of ultra-violet light on solutions of formaldehyde at different concentrations.)

Sugar formation was attempted with 0.001 and 0.0002_5M formaldehyde solution with neutral and basic alumina as the catalysts. No sugars were found after several weeks of continuous refluxing (IVa,b). It would seem that a relatively high concentration of formaldehyde is required before inorganic catalysts can catalyse the formose reaction ($>$ 30ppm. by weight).

Too much formaldehyde, on the other hand, can also be disadvantageous. Equal masses of alumina and formaldehyde were heated together in a relatively small volume of solution. No formose was produced after 24 hours of refluxing (IVc).

Pfeil showed that an excess of formaldehyde favoured the Cannizzaro reaction.[78]

iii. The Effect of the Atmosphere.

Both types of carbon-containing atmosphere that we mentioned earlier have components which can alter the pH of the environment. The reducing atmosphere has ammonia, the oxidising carbon dioxide.

Formaldehyde solution was heated in the presence of alumina under atmospheres of nitrogen, air (oxygen) and carbon dioxide. Inspection of the products by chromatography showed that there was no significant difference in the distribution of sugars (Va,b,c).

Ammonia and formaldehyde react together to form hexamethylenetetramine. Both hexamethylenetetramine and an ammonia-formaldehyde mixture failed to give sugars after five hours at 100° (Vd,e) in the presence of neutral alumina. Neither were sugars formed when hexamethylenetetramine was left in contact with neutral and basic alumina for five months at room temperature (Vf,g).

Hence, of the various atmospheric components that have been examined, ammonia was the most important: pH changes caused by carbon dioxide and the oxidative properties of air do not affect the formose reaction. We might expect that a molar deficit of ammonia (relative to formaldehyde) might permit the formose reaction, by rendering only some of the total amount of aldehyde inactive. (This happens with amino acids, as we shall see shortly.)

iv. The Effect of Sugar.

In the presence of sugar the lag period is reduced or eliminated (Chapters 1,2) and the reaction can occur at a lower pH (Chapter 3). An alternative to the primary reaction has been provided.

Fructose-containing formaldehyde solution was left in contact with neutral and basic alumina at room temperature for five months

TABLE VII

REARRANGEMENT PRODUCTS OF SUGARS ON ALUMINA

STARTING SUGAR	PRODUCTS C_3	C_4	C_5	C_6	INITIAL pH	FINAL pH	FINAL COLOUR OF ALUMINA
D-Glyceraldehyde	+	+	?	++	7.7	7.3	yellow
1,3-Dihydroxyacetone	+	+	?	+	7.5	7.3	yellow
D-Ribose	+	+	+	+	7.6	7.3	yellow
D-Glucose	+	+	?	+	7.6	7.3	yellow
D-Fructose	+	+	?	+	7.7	7.3	yellow
Sucrose	-	-	-	-	7.7	8.6	white

Almost all of the formaldehyde remained at the end of this time (VIa,b,c).

Although large amounts of glucose have been said to cause inhibition of the formose reaction[82], the addition of a five-fold molar excess of the same sugar to our alumina system caused only an increased rate of formaldehyde uptake (Chapter 2). Similarly, an alumina-formose mixture was able to consume further portions of formaldehyde without inhibition (Chapter 2).

v. Rearrangements of the Sugars.

The ease with which 2-deoxyglucose accelerated the formation of sugars from formaldehyde illustrated the facility with which retroaldolisation occurred (Chapter 2). We would expect the same of "normal" sugars. The families of sugar observed after refluxing sugar solutions in the presence of alumina are recorded in Table VII.

During these reactions the pH fell, probably because of caramelisation. Sucrose was the exception, giving no sign of lower molecular weight fragments: an increase in the pH due to dissolution of the alumina supports this finding. The difference between sucrose and the other sugars suggests that the ability to form an enediol is all important. As a control experiment (VII ') each sugar was refluxed with formaldehyde solution at pH 7.4 in the absence of alumina. The same degradation patterns were not formed, although there was a slight suggestion of isomerisation and retroaldolisation. We can conclude from this that the presence of alumina is important for this process, the alumina perhaps providing co-ordinating centres for the enediol ligands.

vi. The Effect of Cyanide.

Hydrogen cyanide often accompanies the formation of formaldehyde in the electrical discharge type of experiment (e.g. Miller and Urey) As we have seen cyanide ions can significantly alter the pH characteristics of the formaldehyde condensation (Chapter 3) although the kinetics on alumina are not altered (Chapter 2). We should expect cyanide ions to alter the distribution of formose sugars through their ability to convert aldehydes and ketones into cyanohydrins. This is observed (VIIIa). At the start of the reaction 10% cyanide (molar, relative to formaldehyde) was added. The proportion of cyanide relative to the carbonyl group increases as the reaction progresses, approaching unity if we assume a small loss of sugar due to caramelisation and formaldehyde due to the Cannizzaro reaction.

The spectra of products differ depending on the presence of cyanide ion. There is a much greater preponderance of hexose (probably aldo-), and much less pentose and triose when cyanide is present. A large colourless spot in the latitudes of triose was found: it had been observed before, when the aniline oxalate spray had been used, but in lesser amounts.

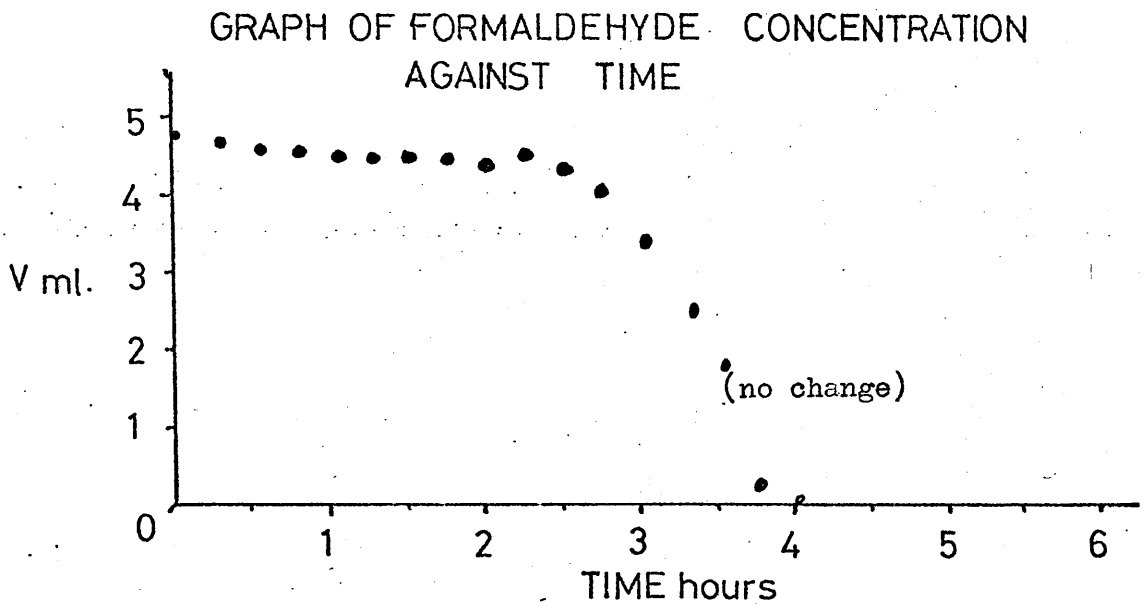
After five months most of the formaldehyde remained in a cyanide-formaldehyde mixture; neutral and basic alumina were the intended catalysts.(VIIIb,c).

vii. The Effect of Acetaldehyde.

Acetaldehyde does not accelerate the formose reaction (Chapter 2). It may , however, participate in aldol condensations. In this way 2-deoxysugars can be formed.

Oro found that glyceraldehyde and acetaldehyde condensed to 2-deoxyribose in the presence of various bases: MgO, $Ca(OH)_2$, and $Ba(OH)_2$ were very efficient catalysts (that with lime went to completion in a few minutes at 0°), but monovalent bases, including ammonium hydroxide, were only moderately active.

Fig. X



30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA + 1 mg. glycine

The destructive influences of the bases was in the reverse order.[255]

Acetaldehyde did not initiate the formose reaction at room temperature within five months (IXa,b).

Acetaldehyde and formaldehyde are able to form pentaerythritol by a series of aldol condensations followed by a cross-Cannizzaro reaction.[256]

viii The Effect of Amino Acids.

Compounds possessing an amino group (such as amino acids and the nucleic acid bases) will react with formaldehyde in the usual manner. It would be expected that only when a molar excess of formaldehyde was present, would the formose reaction occur (on the basis of a 1:1 ratio of reacting aldehyde and amino groups: this will not be valid where there is a 1:2 ratio, such as in protein cross-linking). The effect of ammonia has already been discussed.

Addition of a molar deficit of glycine to formaldehyde did not significantly alter the shape of the formaldehyde consumption curve (Fig.X).

Conclusions.

Many factors have to be taken into consideration when the probability of the formose reaction on the prebiological earth is being assessed. The availability of formaldehyde is the first of these, the remainder concern the factors which influence the formation of sugars from formaldehyde. These factors may be classified according to their ability to affect the formose reaction itself, or to react with formaldehyde. Examples from the first class are temperature, and pH: examples from the second class are ammonia and cyanide. Local production of

formaldehyde could overwhelm the second class of formose antagonist but would leave the first unchanged.

- - - - -

There is another application of the formose reaction to the origin of life theories. It may serve as a model for a primitive metabolism. The next and final chapter is devoted to this.

ANILINE OXALATE SPRAY - COLOURS GIVEN BY VARIOUS SUGARS AND OTHER COMPOUNDS.

COMPOUND	COLOUR((DAY-LIGHT)	COLOUR (ULTRA-VIOLET LIGHT 254nm.)	$R_{glucose}$
Formaldehyde	yellow	yellow	
Methanol	-	-	
Acetic acid	-	-	
Formic acid	-	-	
Glycolaldehyde	v. wk. brown	light blue (streak)	2 streak
D-Glyceraldehyde	v. wk. brown	light blue (streak)	1.2-1.9
1,3-Dihydroxyacetone	orange	orange	1.78
Erythrose (?)	yellow	blue	1.5-1.7
Threose (?)	yellow	blue	1.3-1.7
D-Ribose	pink	pink	1.38
D-Arabinose	pink	pink	1.16
D-Xylose	pink	pink	1.26
D-Lyxose	pink	pink	1.33
D-Ribulose	pink	pink	1.46?
D-Glucose	orange brown	brown centre: light green edge	1.00
D-Mannose	orange brown	brown centre: light green edge	1.12
D-Fructose	yellow-brown (wk.)	light green	1.15
L-Sorbose	yellow-brown (wk.)	light green	1.10
D-Galactose	yellow brown	brown centre: light green edge	0.94
D-Tagatose	light brown (wk.)	brown centre: light green edge	1.15
Sucrose	light yellow-brown	light brown	0.49?
Furfural	pink	pink	

EXPERIMENTAL

Discussion.

Aniline Oxalate Spot Tests.

Formaldehyde applied to a piece of chromatography paper gives a distinctive bright yellow colour after application of aniline oxalate and heating to ca. 90° for 15 minutes. (The heat treatment may be speeded up by the application of a stream of hot air from a blower. This method is however not siutable for heating chromatograms as an even distribution of heat should be applied.) The colour is quite distinctive from that obtained by similar treatment of other sugars or formose mixtures. It may be used as a rapid qualitative test for formaldehyde.

When samples of formose were being applied to chromatograms, it was not always possible to assess how much was being applied without some independent measurement of the total yield. The Cannizzaro reaction and caramelisation decreased the total yield of sugar. It was therefore useful to have a rapid method of estimating the quantity applied.

Preparation of a series of spots corresponding to the mixtures to be chromatographed on a separate, smaller piece of chromatography paper (filter paper can also be used) and exposure of this to aniline oxalate spray, gave after a few minutes a semi-quantitative estimate of the amount of material already applied to the chromatograms. In the presence of formose the spot test gave a brown colour: the intensity depended on the amount of sugar present. If no sugars were present and formaldehyde remained unchanged, then a bright yellow spot was observed.

This procedure was very useful in the study of the formose reaction where, in the majority of cases, either "no formose" or "all formose" was found. The method had a slight deficiency in that it was able to estimate only <u>total</u> sugar. It could not give an indication

of the number of sugars that were present. This would have been useful as the number of sugars governs to a certain extent how much sugar should have been applied: less formose need have been applied if only one or two sugars were formed. The product was therefore chromatographed on a "trial and error" basis in the hope that it contained an average number of sugars. This was successful in most cases.

Paper Chromatography.

The products of the formose reaction were analysed by paper chromatography on Whatman's No. 1 "Chroma" paper with n-butanol: acetic acid : water as 12: 3: 5 by volume as the solvent. The size of the chromatograms was 10" x 22" or 18¼" x 22½": a perspex template assured even spacing of the spots. Serrations were cut at the bottom of the chromatogram so that the solvent could run off the bottom of the chromatogram without causing lateral movement. The running time was typically 22 hours. The chromatograms were then dried in a current of air at room temperature.

Locating Reagents for Sugars.

A variety of sprays were used at one time or another, but only two were adopted for general use - ammoniacal silver nitrate and aniline oxalate.

i Ammoniacal silver nitrate.

Three solutions were prepared

a. 10ml. saturated $AgNO_3$ in water was added to 1990ml acetone; sufficient water was then added to dissolve all of the nitrate:

b. 10g. NaOH were dissolved in the minimum volume of water and the solution was made up to 2 litres in ethanol:

c. concentrated ammonia solution was diluted to give a solution of 6N NH_4OH in 33% ethanol.

The chromatograms were passed through the first solution and then allowed to dry for a few minutes. After passing through the other solutions in turn it was placed in a water bath. After it had been there for approximately half an hour it was carefully removed before drying in a current of air.

Easily oxidisable substances such as sugars create brown or black spots: the reagent is of high sensitivity but low specificity.

ii Aniline Oxalate.

1 ml. aniline (redistilled over zinc) was added to 99ml 0.1M oxalic acid solution. After shaking crystals of aniline oxalate appeared. The solution was allowed to stand for a few minutes before the crystals were removed by filtration. The resulting solution was clear.

The solution is applied as a spray to the chromatograms,,whc which are then heated at 80 - 95° for approximately 15 minutes. The colours observed with a number of sugars are recorded opposite.

After exposure of the chromatograms to light for 24 hours the chromatograms had deteriorated, the background having taken on a yellow colour and the distinctive colours of the sugars having faded.

Aniline oxalate is more specific than ammoniacal silver nitrate but slightly less sensitive.

pH Determinations on the Rocks.

The pH exerted by each rock was recorded in Table I. Estimates were made to the nearest $\frac{1}{2}$ unit as the rocks were slowly dissolving in the water. The dissolution of alumina in water, and the extreme slowness with which equilibria were attained were mentioned in Chapter 2.

The Experiments.

Ia. The generality of the formose reaction.

6.0g. powdered rock were added to 25ml. distilled water and left for a few minutes before the pH was estimated. 5.0ml. 6 x 0.13M formaldehyde solution ("AnalaR", BDH) were then added giving 30ml. 0.13M formaldehyde solution. The solution was refluxed for 5 hours under nitrogen. After cooling the final pH was determined and the solid removed by filtration. A small amount of colloidal material passed through the filter paper (Whatman's No. 1), but did not cause serious problems. An aliquot of the solution was removed for formaldehyde determination by the chromotropic acid method: the solution was then concentrated by rotary evaporation. Where sugar had been formed the solutions were brown and possessed the odour of caramel. Where no sugars had been formed, the solutions were clear and the concentrate contained a white solid (paraformaldehyde).

Chomatography of the products combined with the various locating agents strongly suggested that sugars had been formed. In all cases there was agreement of the chromotropic acid and aniline oxalate methods of formaldehyde determination.

A formalised chromatogram of the products is given in Table I.

Ib. Extraction of a Selection of the Rocks.

Some of the rocks which were available in quantity were subjected extracted with water for 5 hours at 100°. The solution at the end of this time was analysed as above. No sugars nor formaldehyde were found.

Ic Addition of 1,3-dihydroxyacetone.

0.1mg. 1,3-dihydroxyacetone was added to formaldehyde solution (0.13M) in the presence of slate, horneblende, albite, granite and silica. The procedure was the same as in Ia.

Formose was not detected in any after 5 hours of refluxing at 100°.

Id. Oxides, Hydroxides and Carbonates.

A selection of oxides, hydroxides and carbonates, all of technical grade, were added to formaldehyde solution. The procedure is the same as in Ia. Results are recorded in Table I".

II. The Formose Reaction at a Lower Temperature.

3.0g. powdered rock were added to 15ml. 0.13M aqueous paraformaldehyde solution.in polythene tubes. The mixtures (Table II) were left for 38 months at room temperature with occasional shaking. At the end of that period, formaldehyde was estimated by the chromotropic acid method and the u.v. absorbance 225 --275nm. taken as an indication of the presence of caramel.

III. Altering the Amount of Alumina Relative to a Fixed Amount of Formaldehyde.

Various amounts of alumina (neutral) were added to 15ml. distilled water (see Table III) and the pH was measured after 12 hours. 60mg. portions of paraformaldehyde were then added to each before refluxing for 5 hours. At the end of this time formaldehyde was estimated qualitatively by the chromotropic acid method and the colour of the alumina noted. A final pH measurement was also made.

IV. The Formaldehyde Concentration.

a. 1.5g. n-Al_2O_3 + 30mg. PFA + 4.0l. distilled water.
(i.e. 0.0002_5M CH_2O)

The solution was refluxed for 10 weeks,after which time the alumina was removed by filtration and the solution concentrated. A qualitative formaldehyde test (chromotropic acid) on the concentrate was positive. The alumina was white at the end of the reaction time.

b. 20mg. b-Al_2O_3 + 120mg. PFA + 4.0l. distilled water. (i.e. 0.001M CH_2O).

The procedure was the same as above, with the period of reflux being 6 weeks. A positive test for formaldehyde was obtained at the end of this time. The alumina remained white.

c. 6.0g. n-Al_2O_3 + 6.0g. PFA + 30ml H_2O.

The solution was refluxed for 24 hours. At the end of that time the alumina was white and much paraformaldehyde that had not dissolved remained. There was no browning of the solution Aniline oxalate and chromotropic acid tests for formaldehyde were positive.

V. The Effect of Various Atmospheres.

a-c. 6.0g. n-Al_2O_3 were placed in flasks with 30ml. distilled water. Nitrogen, air and carbon dioxide (a,b,c respectively)were were passed through the flasks for 24 hours.

120mg formaldehyde were added to each flask before the solutions were refluxed for 5 hours. The procedure is otherwise as in Ia. No differences in the sugar distributions were observed by use of the aniline oxalate spray.

d. 6.0g. n-Al_2O_3 + 30ml. solution, 0.13M in CH_2O, 0.90M in NH_4OH

e. 6.0g. n-Al_2O_3 + 30ml. distilled H_2O + 93mg. hexamethylene-tetramine. (effectively 0.13M in CH_2O)

After refluxing for 5 hours formaldehyde remained (chromotropic acid and aniline oxalate tests) in both d. and e..
Control experiments in which d. and e. were repeated in the absence of alumina had the same results.
Chromatography of the products from d. and e. and their control experiments failed to show the presence of sugar.

f. 3.0g. n-Al_2O_3 + 15ml. distilled water + 93mg. hexamethylene-tetramine

g. 3.0g. b-Al_2O_3 + 15ml distilled water + 93mg. hexamethylene-tetramine

THE EFFECT OF REFLUXING VARIOUS SUGARS WITH NaOH-HCl SOLUTION AT pH 7.4

STARTING SUGAR	PRODUCTS C_3	C_4	C_5	C_6
D-Glyceraldehyde	+	?	?	
1,3-Dihydroxyacetone	+	?	?	
D Ribose			+	
D-Fructose		?		+
D-Glucose		?		+
Sucrose				

These mixtures were left for 5 months at room temperature: formaldehyde remained at the end of this time (chromotropic acid).

VIa 3.0g. n-Al_2O_3 + 15ml. 0.13M aq. PFA + 1mg. D-fructose

b 3.0g. b-Al_2O_3 + 15ml. 0.13M aq. PFA + 1mg. D-fructose
The procedure is as in Vf,g. Formaldehyde remained at the end of 5 months.

VII Rearrangement of Sugars on Alumina.

3.0g. n-Al_2O_3 + 15ml distilled water + 60mg. sugar (see Table VI)
The contents of the flasks were maintained at 100° for 4 hours. Chromatography of the products showed the sugar distributions recorded in Table VI. pH measurements were also made.

Control experiments.
Each sugar was refluxed in aqueous solution at pH 7.4 (NaOH-HCl) for 4 hours. The procedure is the same as above. Chromatography of the products showed traces of isomerisation and rearrangement reactions but not to so great an extent as in the presence of alumina (see facing Table)

VIII Effect of Cyanide.

a. 6.0g. n-Al_2O_3 + 30ml. 0.13M aq. PFA. + 13 mg. KCN

The solution was maintained under reflux for 5 hours after which the products were chromatographed, as described earlier. The spectrum of sugars obtained is described in the text.

b. 3.0g. n-Al_2O_3 + 15ml. 0.13M aq. PFA + 6.5mg. KCN

c. 3.0g. b-Al_2O_3 + 15ml. 0.13M aq. PFA + 6.5mg. KCN

The precedure is as in Vf,g. Formaldehyde remained after 5 months had elapsed.

IX a. 3.0g. n-Al_2O_3 + 15ml. 0.13M aq. PFA + 9mg. CH_3CHO.

b. 3.0g. b-Al_2O_3 + 15ml. 0.13M aq. PFA + 9mg. CH_3CHO.

The procedure is as in Vf,g. Formaldehyde remained after 5 months at room temperature.

X. 30g. n-Al_2O_3 + 150ml. 0.13M aq. PFA + 1 mg. glycine.
The procedure for this experiment will be found in Chapter 2.

CHAPTER 6

THE FORMOSE SYSTEM AS A POTENTIAL PHENOTYPE

Introduction.

The current theory of Chemical Evolution involves nucleic acid as the first and only genetic material. It is easy to see why this is so. It is because nucleic acid is today's genetic material, and because no other genetic material is known. It would not be correct to assume from this that nucleic acid is the only kind of molecule that may contain information. Many molecules are potential information holders: protein, polysaccharide and clay might be taken as examples.

Nucleic acid may not have been the first genetic material. The possibilities of clay as an information carrier have been discussed by Cairns Smith.[257] Here we are dealing with a function that has to be satisfied (the preservation and propagation of useful information), and are not concerned with the involvement of specific molecules (nucleic acid).

The mere existence of an information-containing molecule does not make it genetic. Just as a spool of magnetic computer tape needs a computer, so does the information-containing molecule need a means of expression. Also, it is not possible to tell what information is possessed by the molecule simply by looking at it: molecules which carry "nonsense" will only be distinguished from those which carry "sense" in an environment suited to the latter. In another environment these roles could be reversed.

In an organism the manifestation of the gene is called the phenotype, the body. The necessity for genotype-phenotype

matching has also been discussed by Cairns Smith, with particular reference to the evolution of genetic control from the primitive environment.[258] The most primitive organisms will have an elementary form of matching: their biochemistries will reflect the events in the primitive environment. It is there that we should look for physico-chemical systems, before attempting to progress to simple organisms.

Discussion.

The appearance of genetic control represents one of the greatest steps in the origin of life. It is then that natural selection begins to operate. (One can envisage a form of natural selection before this that operated on unique individuals. Any information that they had gained would be easily lost, because of their inability to disseminate it. When there is a community of similar but self-reproducing units, chance events will not have such a serious effect.)

How, then, is genetic information transmitted to the phenotype? Surely it must be by the formation of physical or transient chemical bonds. (If the bonds are not transient, they are permanent and are then considered as a part of the gene.) In this respect the genetic material behaves like a catalyst.

The natures of the genotype and the phenotype are different. The former is static (except for its reproductive capacity): the latter is essentially dynamic, and must be so in order to manifest the genotype. In modern organisms genetic reproduction occurs within an existing, matched phenotype (the "parent"). In the primitive environment the first gene will be unable to rely on such systems.

The requirements for the primitive gene have been listed.[257] It will have a low information content, but a large potential. It will be stable, have the capacity for accurate replication

and still be able to mutate occasionally. The information that it holds must have survival value.

The communication of genetic information probably occurs by surface contact. DNA has a large surface area, and its mode of replication is well known. The primitive gene will have to utilise its surface, too, but it need not have had such a large surface-to-mass ratio as in the present genetic material. Selection pressures would, however, operate to reduce the size of the genetic material to a minimum. In the first "organism" the monomers of the genetic material would not necessarily have had to be made inside a sophisticated phenotype: they could have been supplied by the environment.

What are the requirements of the primitive phenotype? It must be dynamic, and have the ability to change according to changes in the genotype (subject to natural selection). The phenotype of the first organism may not have been as discrete as in evolved organisms. There may have been a communal phenotype. 258 The primitive phenotype would have to rely on the environment for its supply of high energy compounds (food).

Which came first, then, the genotype or the phenotype? Of course, no phenotype is possible without a gene, but there are systems which could be regarded as potential phenotypes. (The formose system is an example.) Let us return to the original question. A number of alternatives are possible. They have been illustrated opposite. That a "naked gene" sought a matched, dynamic system under catalytic control, and replaced the catalyst is one possibility. That the catalyst of a dynamic system acquired the ability to replicate is another. Thirdly, a latent gene might have established its own phenotype by using one or two specific molecules from the environment as "food".

We can use the formose system for examples of each type.

Fig. I

POSSIBLE METHODS BY WHICH GENETIC CONTROL WAS ESTABLISHED

1. $(S + C) + G_l \longrightarrow (S + G_a) + C$

2. $(S + C) \longrightarrow (S + G_a) \therefore C \rightarrow G$

3. $G_l + M \longrightarrow (S + G_a) \therefore M \rightarrow S$

S - DYNAMIC SYSTEM.

C - CATALYST FOR S.

G - GENE

M - MONOMER FOR S.

a - ACTIVE

l - LATENT

Clay is a known formose catalyst,[119] and its capacity as a primitive genetic material has been discussed.[257] Let us use it as a genetic material for the three systems.

If clay, G_1, replaces the formose catalyst, C, and takes over the control of the formose system, then we have the situation that obtains in "1". Suppose clay catalyses the formose reaction in an environment devoid of clay monomers: the clay is behaving as a catalyst, C, only. If these monomers are then supplied, the clay will be able to replicate and this satisfies the second equation. The third equation will be satisfied if the clay is able to initiate and continue the formose reaction, using only formaldehyde. (It differs from the first case, in that the material G_1 cannot initiate the formose reaction, but can only continue it.) In this respect we can see that the clay behaves like a virus, being able to withstand periods of inactivity: but, unlike a virus, the clay can create a new system from formaldehyde only -the virus relies on the provision of a sophisticated phenotype from another organism in order to propagate (type 1 ?).

The scope for variation within the formose system is great. There are a large number of polyhydroxyaldehydes and ketones, both straight chain and branched, which can be formed by the aldol condensations of sugars. As we saw in the previous Chapter, the mixtures of sugars arising from rock catalysis of the formose reaction are varied. It is perhaps surprising that they are not more varied. Table A shows diagramatically many of the structural isomers of the polyhydroxyaldehydes and 2-, 3-, 4-ketones of empirical formula CH_2O, containing up to seven carbon atoms. For reasons of space, branches with more than one carbon unit have been omitted.

TABLE A

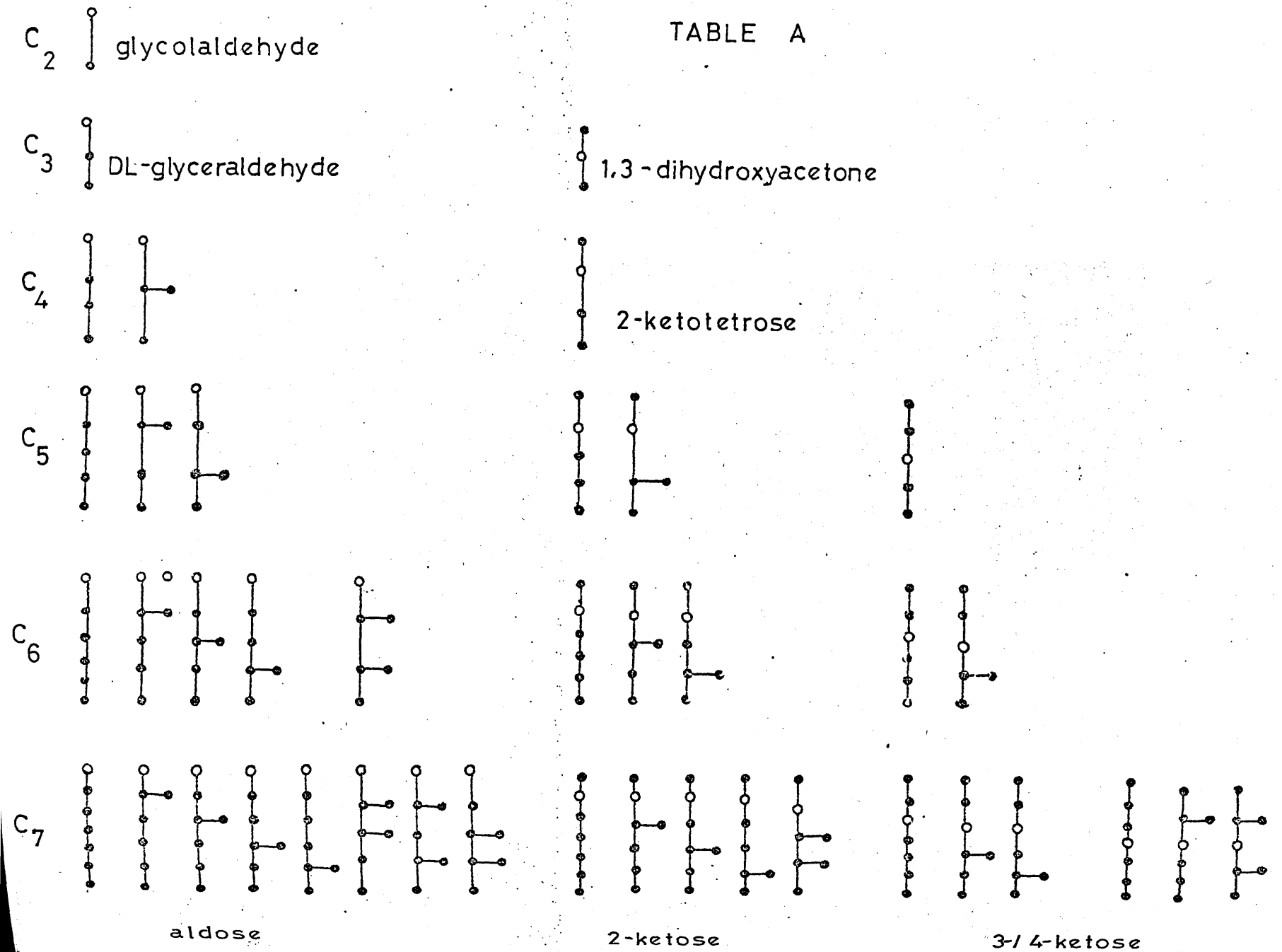

C2
glycolaldehyde
C3
DL-glyceraldehyde
1,3-dihydroxyacetone
C4
2-ketotetrose
C5
C6
C7
aldose
2-ketose
3-/4-ketose

TABLE B

CARBONYL ISOMERISATIONS

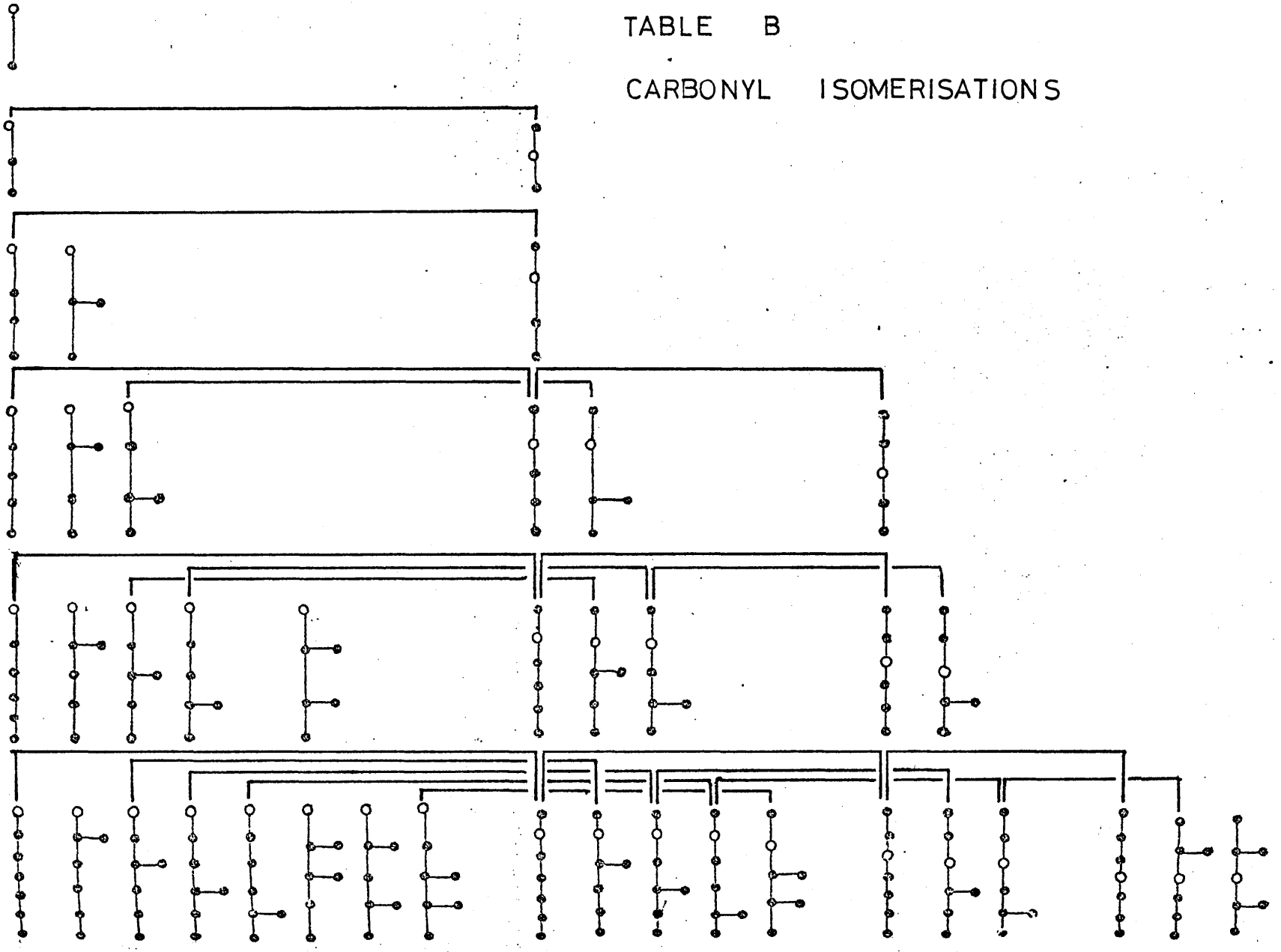
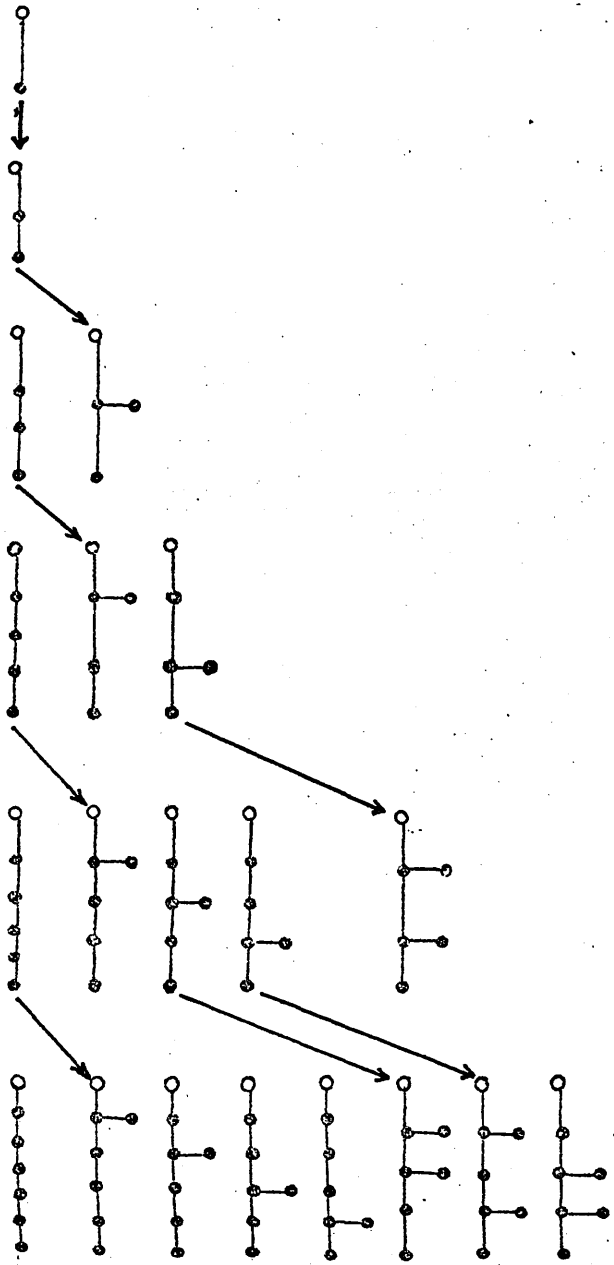


TABLE C

UPTAKE OF FORMALDEHYDE BY ALDOL CONDENSATION



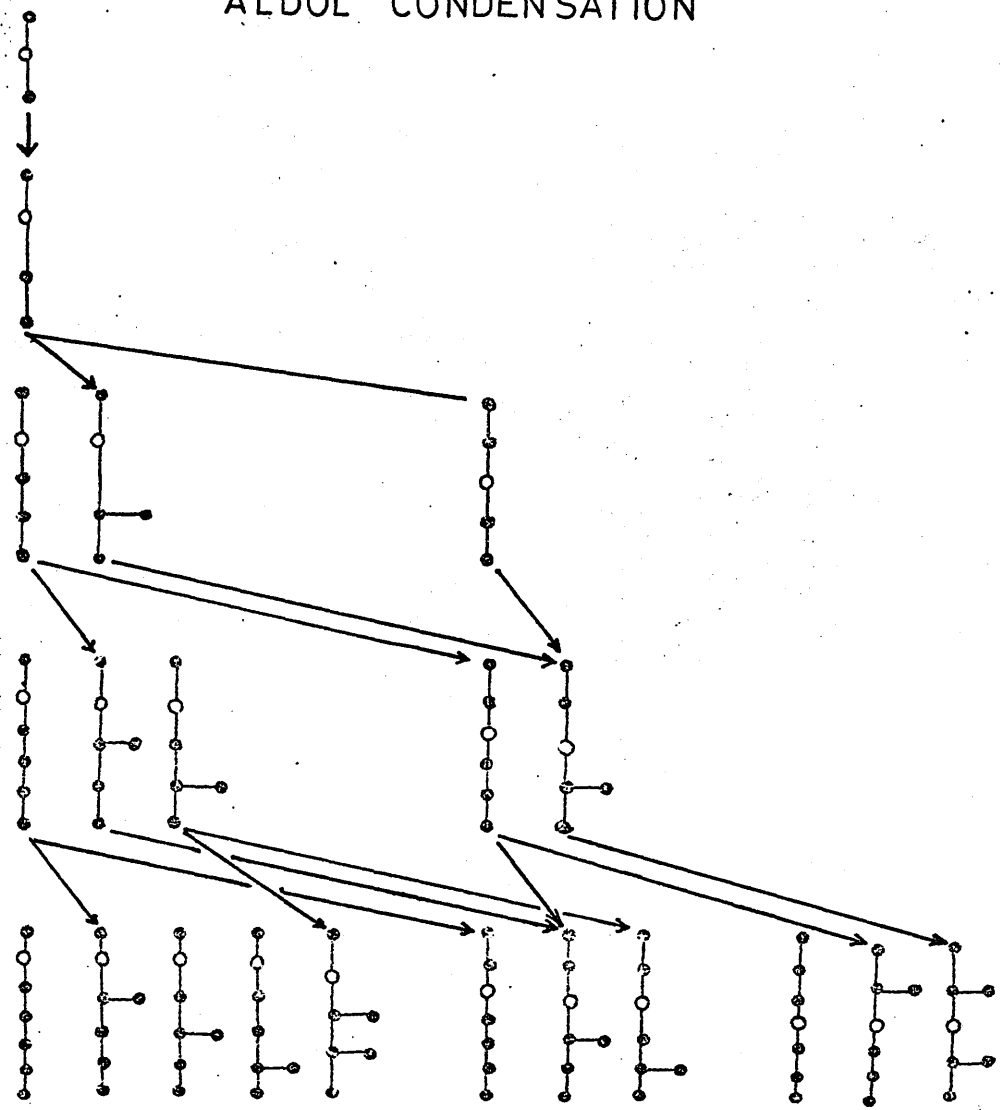
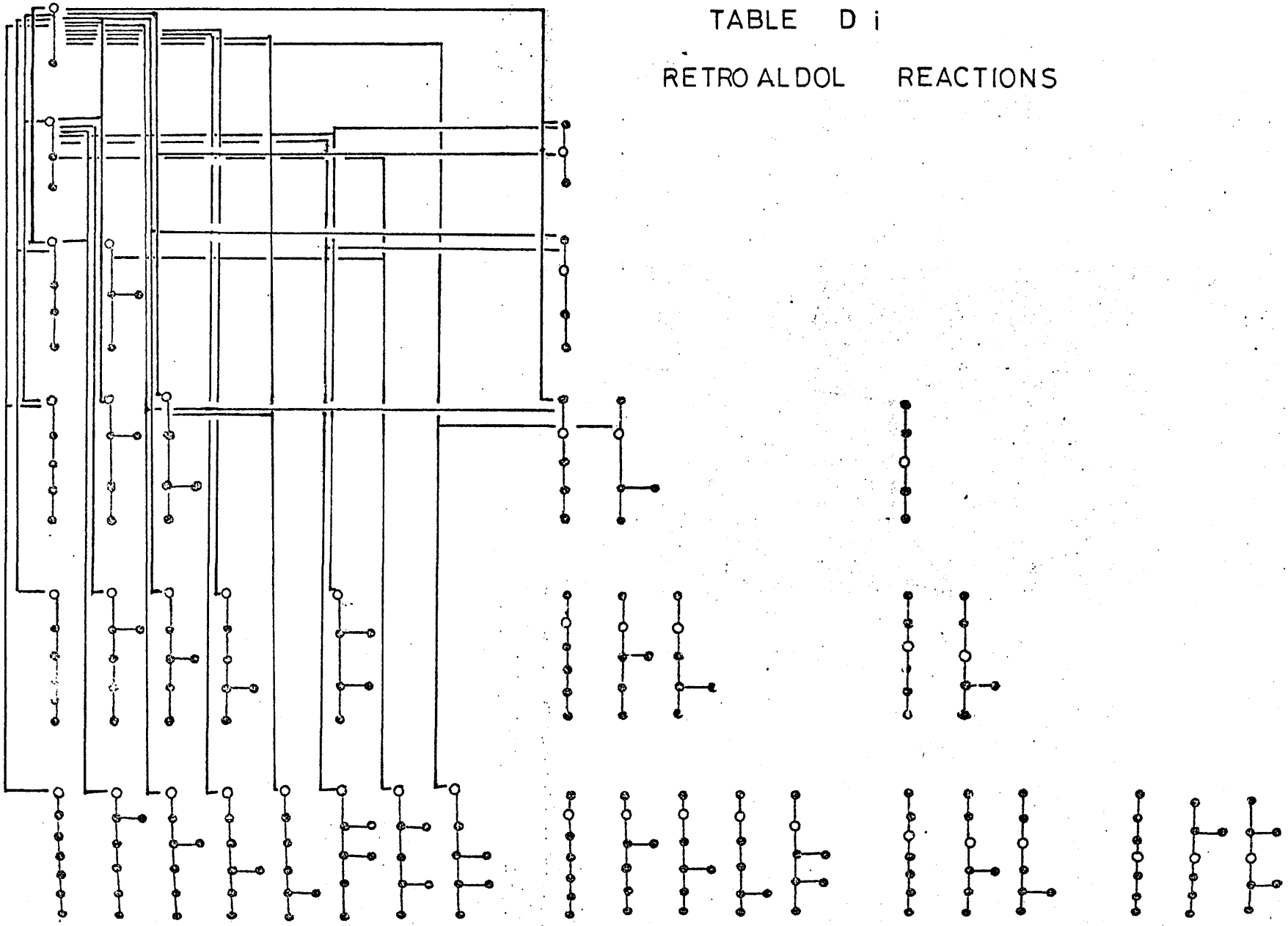


TABLE D i

RETRO ALDOL REACTIONS



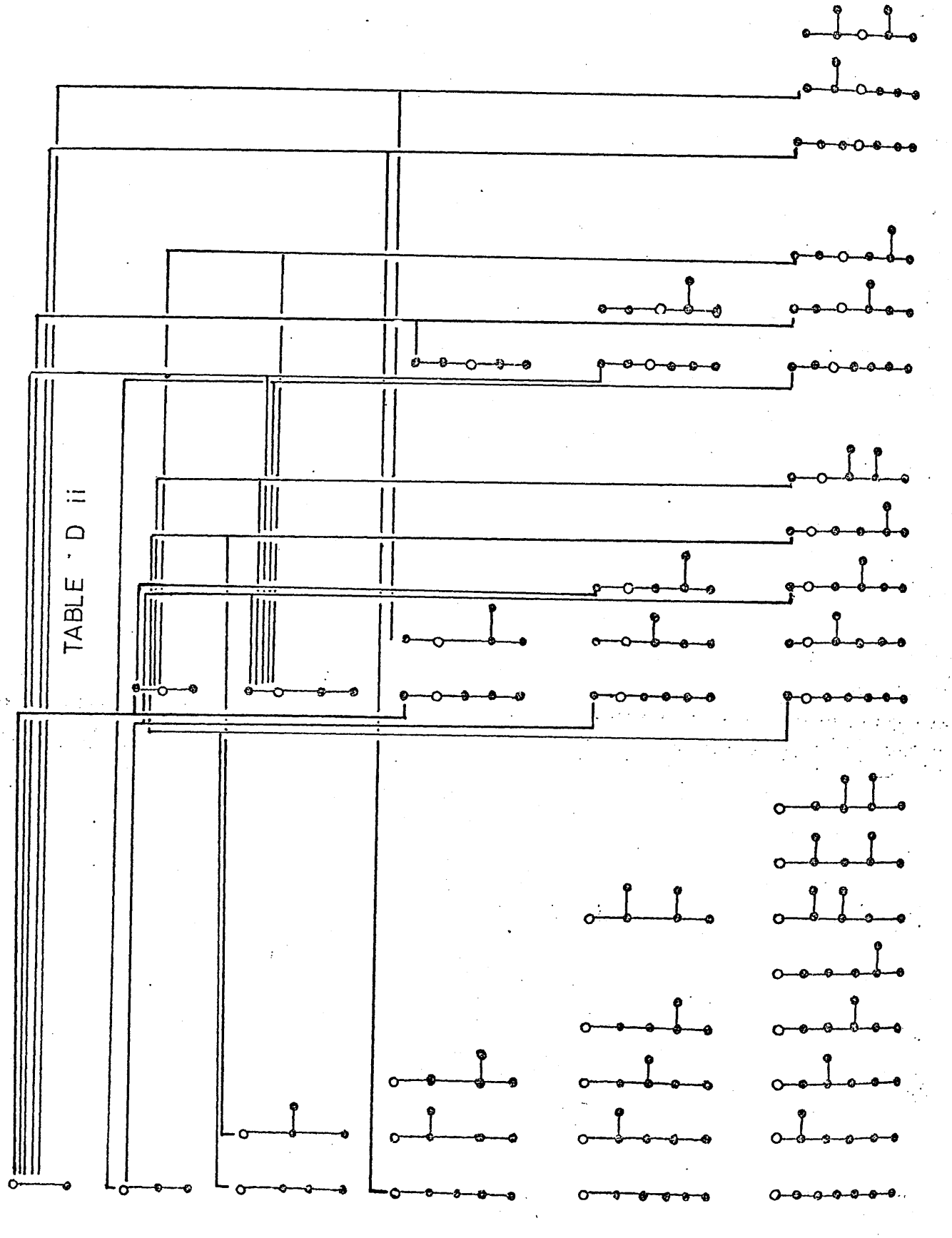
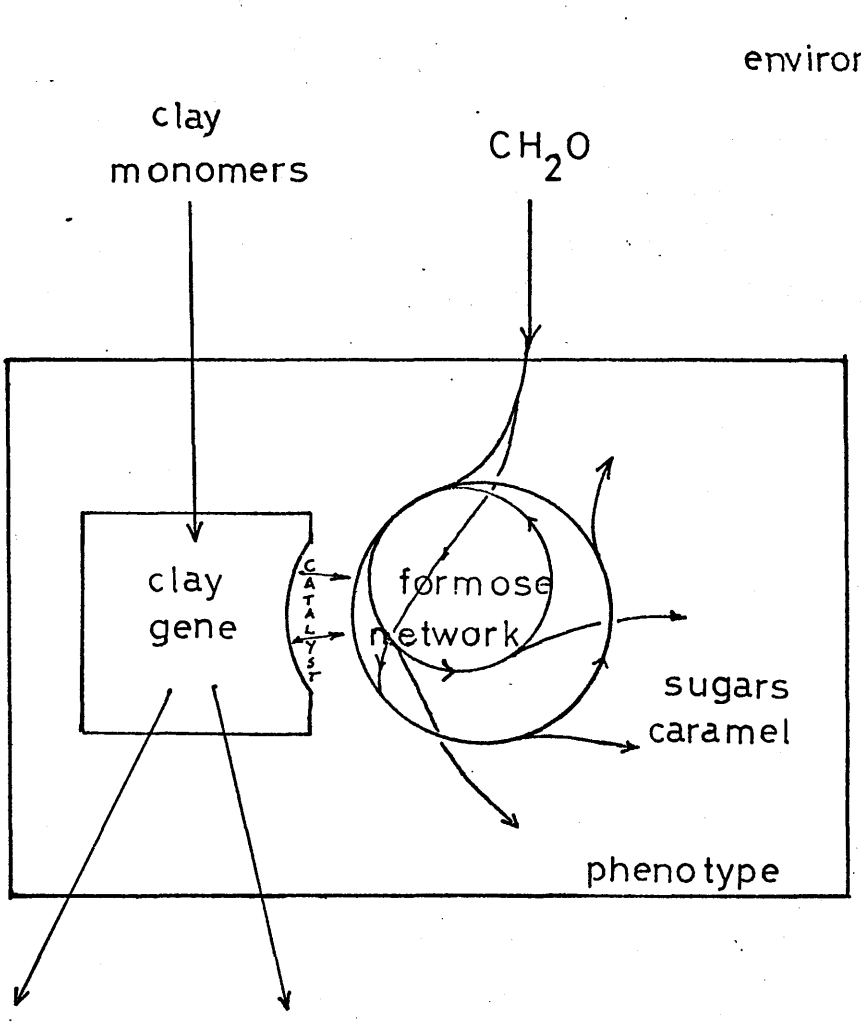


TABLE ' D ii

Fig. II


environment
clay
monomers
CH_2O
clay
gene
CATALYST
formose
network
sugars
caramel
phenotype

The "o"s in the diagram represent aldehydes or ketones, depending on their position. The "•"s represent $-CH_2OH$, -CH.OH or -C.OH depending on their position. Table A serves as a guide to the understanding of the abbreviations: several sugars have been named. It should also be remembered that the stereochemistry of the hydroxyl groups is not implied in the diagram e.g. aldohexoses are represented as one unit.

Table B shows all the carbonyl isomerisations that are theoretically possible in this scheme. Table C shows the products of these compounds after aldol condensation with formaldehyde. Tables D(i) and D(ii) show the products of retroaldolisation that do not involve formaldehyde. (Formaldehyde retroaldolisation products will be found by reversing the arrows in Table B.) For the sake of clarity the retroaldolisation processes have been divided into the two D tables.

If we wish to have an impression of the total number of pathways open to straight- and branched-chain sugars in the formose system, we should consider all of the reactions in Tables B, C, D and add to these aldol condensations of those compounds which lead to isomers with more than two carbon atoms in a branch. This gives at least an indication of the complexity of the system.

We can see that a number of cycles could be available (the Breslow cycle is only one). It is possible that different catalysts could favour different cycles, perhaps giving a way for changes in the clay gene to manifest themselves.

This clay-formose "organism" has been drawn to show the various processes involved (Fig II). Unlike a modern organism, this one can utilise "raw materials" from the environment directly for its genotype and phenotype, without the necessity for complicated metabolic pathways to change the food into the monomers. The formaldehyde could even be produced within the phenotype of the "organism" making it an autotroph, assuming that part of the gene or phenotype encouraged this. But

distinctions of this kind are difficult because of the large measure of subjective assessment of where the phenotype begins and where it ends.

"When did one of the carbon atoms that you had for breakfast stop being 'environment' and start being 'phenotype'?" [258]

The further possiblilty of a form of self-regulation of pH (Chapter 3) which includes the Cannizzaro reaction and caramelisation should not be overlooked.

Is this formose sytem living? Here we become entangled in the realm of definitions again and a measure of subjective assessment must apply in giving an answer. Certainly, if this system evolved further, an organism with a predominantly carbohydrate phenotype would be a particularly attractive possibility.

TABLE A

PRODUCTS ARISING FROM THE ALDOL CONDENSATIONS OF SUGARS

			Predicted sugar
1.	Formaldehyde and glycolaldehyde.		
	a. C_5, C_{5A}	81	
	b. C_{3A}	46,58	C_{3A}
	c. C_{3K}	96	
	d. C_{5A}, C_{5K}, C_6	97	
2.	Formaldehyde and 1,3-dihydroxyacetone.		
	a. C_5	81	
	b. C_{5K}, C_6	97	C_{4K}
3.	Formaldehyde and erythrose.		
	a. C_5, C_6	97	Branched
4.	Glycolaldehyde only		
	a. C_{4A}	260,262	
	b. C_{6A}	115	
	c. C_4, C_{6K}	270	C_{4A}
	d. C_{4A}, C_{6K}	261	
	e. C_4, C_{5A}, C_{5K}, C_{6A}, C_{6K}	96	
	f. C_4, C_{5K}, C_{6A}, C_{6K}	97	
5.	Glycolaldehyde and glyceraldehyde.		
	a. C_{5A}	115,270,271	
	b. C_{5A}, C_{6K}	81	C_{5A}, and
	c. C_{5A}, C_{5K}, C_{6A}	97	Branched
	d. C_{6K}	264	

TABLE A cont.

6. Glycolaldehyde and 1,3-dihydroxyacetone.			
a.	C_{5A}	270,271	
b.	C_{5K}	270,271	C_{5K}; and
c.	C_{5A}, DK	81	Branched
d.	C_{3K}, C_{5A}, C_{5K}	96	
7. Glyceraldehyde only.			
a.	C_6	97,267	
b.	C_{6K}	259,265,266,276	Branched
c.	C_{6K}, DK	272	
8. Glyceraldehyde and 1,3-dihydroxyacetone.			
a.	C_6	267	
b.	C_{6K}	46,266	C_{6K} , and
c.	C_{6K}, DK	276	Branched
d.	C_5	262	
9. 1,3-dihydroxyacetone only.			
a.	C_6	96	
b.	DK	269	DK
c.	C_6, DK	97	
10. 1,3-dihydroxyacetone and erythrose.			
a.	C_{7K}	270,271	C_{7K}, and Branched
11. Erythrose only.			
a.	C_{5A}, C_{5K}, C_6	97	Branched

TABLE A'

THE DEPENDENCE OF THE RATIO OF KETOHEXOSE TO BRANCHED SUGAR ON THE CATION OF THE CATALYST.[276]

CATALYST	RATIO	CATION RADIUS
LiOH	0.89	0.60 Å
NaOH	1.05	0.95 Å
KOH	1.21	1.13 Å
$Sr(OH)_2$	1.11	1.33 Å
$Ba(OH)_2$	1.17	1.35 Å
Amberlite* IRA 400 (OH^-)	1.40	large

At 25°, 0.05N base, $2\frac{1}{2}$ hours.

* 1 hour

APPENDIX I

ALDOL CONDENSATIONS OF SUGARS

Aldol condensations of sugars are well known in their own right. Here a summary of some of the products, isolated from aldol reactions between sugars up to tetrose, is given - Table A. In many cases the expected products are found, but these are accompanied by a number of other sugars, which are derived by a combination of aldol and retroaldol reactions, and isomerisations of the starting materials and the products. In general the products depend on the time of the reaction, and perhaps, on the catalyst. Condensates with more than seven carbon atoms have not been isolated, due to the thermodynamically favoured cyclisations, which result in deactivation of the carbonyl group to aldol condensation. A small percentage of the aldehydo- and keto-forms will always be present in solution, allowing some condensation to higher sugars, but as the reaction is reversible, no net effect is observed. Nonoses were not isolated from the liquor after the self condensation of glyceraldehyde.[273] Formaldehyde is not a product in these reactions, which suggests that retroaldolisation to formaldehyde does not easily occur.

Does the cation of the base affect the course of the aldol condensation? In the self condensation of D-glyceraldehyde the ratio of the ketohexose to branched sugar was measured for a number of catalysts. It was found that the ratio had a small but real dependence on the cation present, and was in the approximate order of the cation radii. (Table A'.)

Catalysis by hydroxide ion was negligible at pH 7, although a small effect was exhibited by the buffer ions, HPO_4^{2-} and $H_2PO_4^-$ at that pH. Pyridine at pH 6 had a great effect: it was also demonstrable at pH 5.[276] The introduction of groups which cause steric hindrance around the nitrogen, caused a reduction in its catalytic effect.

TABLE A

TABLE OF PRODUCTS EXPECTED FROM ALDOL CONDENSATION OF LOWER SUGARS

(after Ruckert Pfeil & Scharf[115])

	C_1	C_{2A}	C_{3A}	C_{3K}	C_{4A}	C_{4K}
C_1	(C_{2A})	C_{3A}	B	C_{4K}	B	C_{5K}
C_{2A}		C_{4A}	C_{5A}	C_{5K}	C_{6A}	$C_{6\text{-}3K}$
C_{3A}				C_{6K}	B	C_{7K}
C_{3K}				C_{6KD}	C_{7K}	B
C_{4A}						$C_{8\text{-}4K}$

B - branched sugar

D - dendroketose

Ruckert, Pfeil and Scharf composed a table of the products expected from aldol condensations of lower sugars (Table A"). They have classified only the polyhydroxy compounds which have straight chains. The remainder, apart from dendroketose, have been classified only as 'branched'. A considerable number of sugars with straight and branched chains can be formed by aldol condensation, depending on which molecule is the nucleophile and on which side of the carbonyl group the condensation is taking place.

An indication of the complexity of the products that can arise from aldol condensation of lower sugars is given in Chapter 6: here only retroaldolisation processes have been recorded as the table does not include compounds with side-chains containing more than one carbon atom. Aldol condensation products of various sugars and formaldehyde were also included. Again, we should remember that this table does not take account of the stereochemistry of the hydroxyl groups.

APPENDIX II

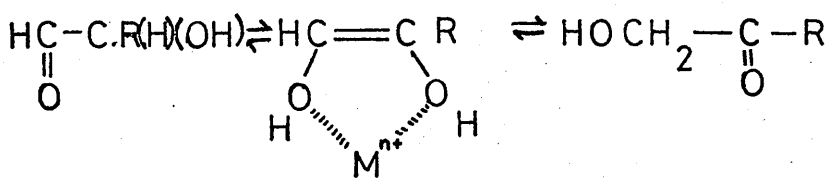
ISOMERISATION AND ENOLISATION OF SUGARS

The interconversion of C-2 epimeric aldoses and the corresponding 2-ketose is a well known reaction (the de Bruyn - van Ekenstein transformation), and is used as a preparative method. It has been fully reviewed elsewhere,[296,297] so only a brief summary is given here.

1,2-enediols are intermediates in the interconversions in most cases; only rarely has the participation of the 2,3-enediol been reported.[273,296,297] Sodium, barium and calcium hydroxides and organic beses, such as pyridine, are the most usual catalysts, for the reaction. Polyvalent ions, such as calcium, help to stablise the enediols (see below).[51,298]

The possiblilty of hydride transfer rather than enolisation as the mechanism of interconversion has also been entertained.[298]

Isomerisations of the carbonyl group have been illustrated in Chapter 6.



SOME OF THE VOLATILE COMPONENTS OF CARAMEL

Aldehydes and ketones:

acetaldehyde, propionaldehyde, n- and iso-butyraldehyde, n- and iso-valeraldehyde, acetone, methyl ethyl ketone, diethyl ketone, methyl propyl ketone.

Esters:

methyl formate, methyl acetate, methyl propionate, methyl n- and iso-butyrate, methyl n- and iso-valerate.

Other compounds:

methanol, ethanol, acrolein, furan, 2-methylfuran.

ALSO FOUND:

succinic, fumaric, furanoic and laevulinic acids.

APPENDIX III

CARAMEL

Formose formation is often accompanied by a smell - but fortun ately it is the pleasant one of heated sugar. The solution becomes yellow or brown, depending on the extent of caramelisation. The extent of destruction of the sugars in an aqueous medium depends on what cations and anions are present. Treatment of 10% glucose solutions with caustic soda, lime and baryta resulted in 100, 97 and 53% destruction respectively after three hours at 100°.[277] Inorganic carbonate accelerated the destruction of glucose while sulphate and phosphate had the opposite effect.[278] This may partly be due to the pH, as the extent of colour formation increases as the pH rises.[279,280] High temperatures favour caramelisation.[120,277]

The presence of formaldehyde may inhibit colour formation,[281] but in the formose reaction this is not serious as Mizuno's work has shown (Chapter 1).

Caramel is a complex mixture of compounds. Some of the volatile components have been identified (opposite); the products depend partly on the pH.[284]

1,3-DIHYDROXYACETONE / FRUCTOSE —(pH 10-12)→ ACETOL, DIACETYL.

1,3-DIHYDROXYACETONE / FRUCTOSE —(pH 8-9)→ less ACETOL, DIACETYL, METHYLGLYOXAL

Sugars may also be destroyed by ultra-violet and γ- irradiation,[285] leading in some cases to formaldehyde and dihydroxyacetone.[285,286,287]

APPENDIX IV

THE CANNIZZARO REACTION

The Cannizzaro reaction often accompanies the formose reaction. Irreversible, it is experienced by those aldehydes which lack a hydrogen atom on the carbon α- to the carbonyl e.g. benzaldehyde, furfuraldehyde, and, of course, formaldehyde. (Formaldehyde is somewhat unique as it does not even have an α-carbon.) The possession of an α- hydrogen atom leads to aldol condensation. A cross-Cannizzaro reaction between the aprotic aldehyde and another aldehyde (either aprotic or aldol-type) is possible, with the former, the hydride donor, undergoing oxidation. An internal reaction occurs in glyoxal.[293]

The mechanism of the reaction is still the subject of study: we shall examine the data with special regard to formaldehyde.

$$2\ CH_2O + H_2O \longrightarrow H.COOH + CH_3OH$$

Catalysts.

The reaction usually is carried out in homogeneous, alkaline solution (aqueous or alcoholic),[133,293] but it may be done in acid.[292,294] A number of inorganic hydroxides have been used as catalysts for the reaction, but reports vary as to which is the "best". With formaldehyde as the substrate, lithium and sodium may be better than thallium ions,[93] or calcium ions may be better than thallium, barium, lithium, sodium, potassium and tetramethylammonium, respectively.[78,82] The rate of the reaction (with benzaldehyde) increases on going down the hydroxides of Group I.[295] Detailed examinations of the sodium[288,289,290] and calcium[131,133] hydroxide catalysed reactions have been made, with formaldehyde. The catalysts for the reaction are similar to formose catalysts. Organic bases are not good Cannizzaro reaction catalysts (Appendix V).

Reaction Order.

The reaction order is subject to considerable variation, ranging from first order in aldehyde (in the presence of an excess of aldehyde and dilute hydroxide),[78] to third order (second in aldehyde, first in hydroxide),[289,290] or greater,(where the hydroxide contribution may rise to almost second order [78]). Thus both the hydroxide ion and the aldehyde are involved in the rate determining step.

Activation Energy.

The Arrhenius activation energy of the reaction (NaOH catalysed) involving formaldehyde was found to be 22,320 cal. mole^{-1}, being increased by solvation of the aldehyde, or by strengthening of the aldehydic hydrogen bond in other aldehydes.[289]

Isotopic Studies.

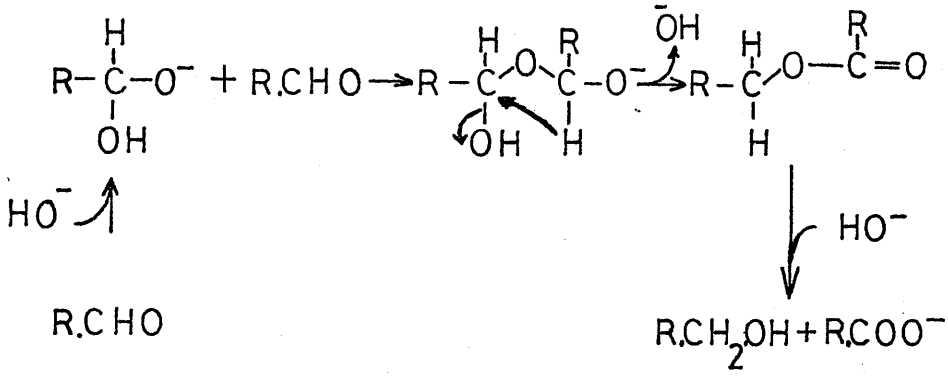
When the reaction is carried out in deuterium*, no solvent is incorporated into the products.[78,293,294] Kinetic isotope effects indicate that the rate determining step involves the cleavage of the aldehydic hydrogen bond.[294] The possibility of an intramolecular reaction has been rejected.[295]

The Balance between the Cannizzaro Reaction and the Formose Reaction.

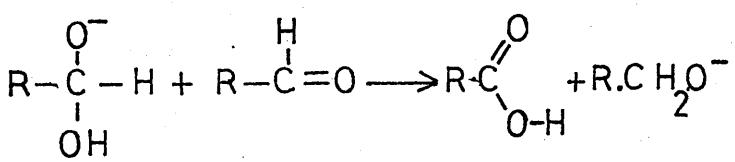
It is obvious that the two reactions favour similar conditions. There is some evidence for "switching" from one to the other. In sodium hydroxide solution from 40 - 60° the Cannizzaro reaction predominates: if the temperature is increased to 70°, the formose reaction is more important.[288,289] A ratio of Tl^+: CH_2O of 1:44.5 (i.e. an excess of aldehyde) causes 100% Cannizzaro reaction: a 1:1.3 ratio results in formose only.[82] It is also possible that a minimum concentration of catalyst is necessary before sugar formation can commence.[77,131]

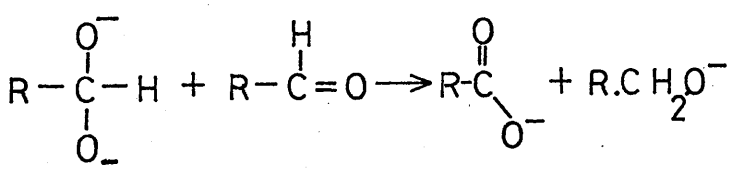
*oxide

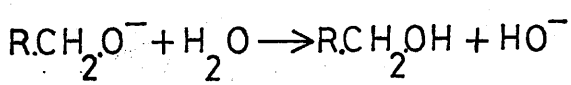
a. Hemiacetal Mechanism



b. Hydrate – Hydride Mechanism

I 

II 



Mechanism.

A number of mechanisms for the Cannizzaro reaction have been proposed. They come into two classes depending on whether they involve the participation of metal ions. (We shall be concerned only with the ionic mechanisms. homolytic reaction may occur on nickel.[293])

Two types of mechanism have been postulated for the sodium hydroxide-catalysed reaction:

a. the hemiacetal mechanism (also called Lock's mechanism,[133] and Geissman's mechanism[295]).[290]
b. the hydrate-hydride mechanism (also called the Ingold mechanism,[290] or the Hammett mechanism.[295] March's mechanism[133] is the same, without the inclusion of the hydrate dioxide ion).

These mechanisms are given on the facing page.

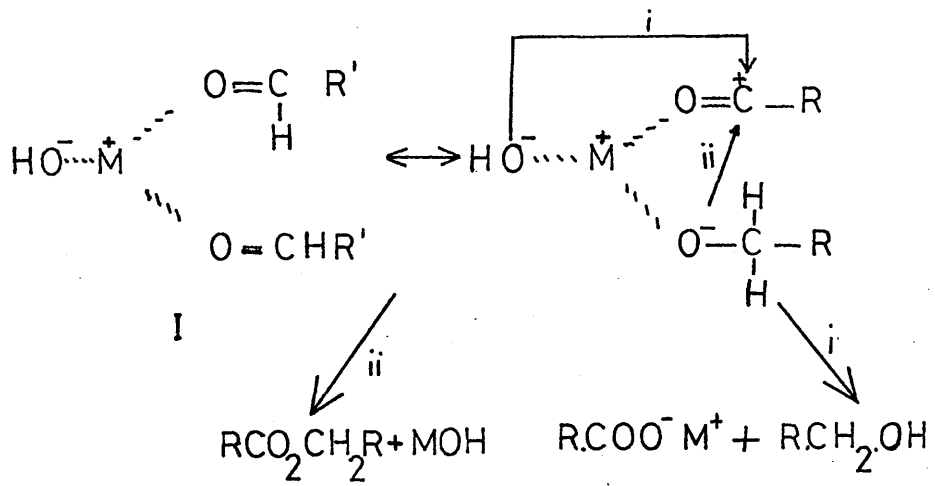
The hemiacetal favour has been dropped in favour of the second pathway. Martin's evidence supports the hydrate-hydride mechanism as it explains why there can be first and second order involvement of the hydroxide ion.[290] Shapira also supports mechanism "b".[133]

In 1951 Pfeil carried out an intensive examination of the role of metal ions in the Cannizzaro reaction.[78,82] He came to several conclusions:

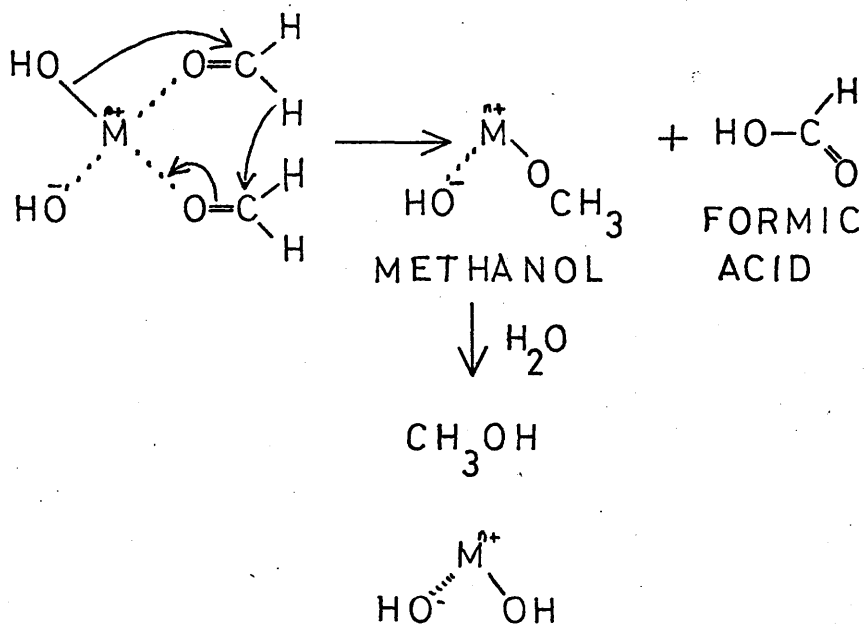
1. the solvent does not participate in the reaction;
2. the reaction velocity in very dilute alkali and in an excess of aldehyde is first order in aldehyde, changing to second order in aldehyde and almost second order in hydroxide in moderately concentrated alkali;
3. the unimolecular region of concentration (2) varies with the cation;

PFEIL'S MECHANISM FOR THE CANNIZZARO REACTION

(See also formose mechanism)



RUNGE AND MAYER'S MECHANISM FOR THE CANNIZZARO REACTION



4. the rate constant for the hydroxides is not proportional to their basic strength - they are, in decreasing order of effectiveness in promoting the Cannizzaro reaction

$$Ca^{++} > Tl^{+} > Ba^{++} > Li^{+} > Na^{+}, K^{+} > Me_4N^{+} \quad ;$$

5. a high aldehyde conentration and a ratio of $Tl:CH_2O$ of 1:44.5 causes complete Cannizzaro reaction, but a 1:1.3 ratio gives only formose;
6. the addition of neutral salts containing the same cation as the base increases the rate of the Cannizzaro reaction; and
7. iso-propanol, tetrahydrofuran and dioxane increase the rate; methanol, ethylene glycol and ethanol decrease it.

He suggested the chelate mechanism on the opposite page, which involves unhydrated formaldehyde.

Complex I is favoured over the formose adduct (Chapter 1, Mechanism) when there is a high formaldehyde to metal ratio. Hydride transfer occurs within this complex to give an acylium ion and an alcoholate. The ease with which this happens depends on the cation of the complex. Two possibilities then arise: the acylium ion reacts with the hydroxide to form the acid, or an ester is formed. There is a similarity between the second part of Pfeil's mechanism and the hemiacetal mechanism, but there is a difference in the time of hydride transfer.

The addition of neutral salts provides a greater concentration of cation - formaldehyde units, which only need hydroxide to change to complex I. Alcohols solvate the aldehyde and so alter the rate by effecting the concentration of available free aldehyde.

Runge and Mayer provide a similar mechanism.[122]

TABLE E.

PATENTS RELATING TO FORMOSE

YEAR	PATENTEE(S)	CONDITIONS	PRODUCTS	REFERENCE
1938	Prudhomme	alkaline earth oxides and rays of luminous energy	sugars up to hexose	60
1939	Taylor, Woolcock. Tyrer, & I.C.I. Ltd.	oxide of Mg, Ca, Sr, Ba, Sn, Pb	C_2, C_3, C_4 : aldose and ketose	61
1940	Handford, Schreiber	Pb - metal, oxide, hydroxide, nitrate, formate Sn - metal, chloride, formate Ca - chloride, formate, oxide BaO, MgO, $ThNO_3$ + G2, G3, DHA, E, Fru, Glu, ascorbic acid, benzoin, reductone	hydroxyaldehydes and ketones, and their hydrogenated products;	62
1942	Handford, Schreiber	similar to 62	continuous process	64
1942	Lorand	hydrogenation of formose		65
1942	Lorand	lead oxide, hydroxide, carbonate, formate, acetate, palmitate, stearate, propionate "uncleaned strips of lead" Zn, Mg oxide, hydroxide	C_2, C_3, C_4 sugars	66

TABLE E cont.

YEAR	PATENTEE(S)	CONDITIONS	PRODUCTS	REFERENCE
1943	Scheonemann, Apel, Berger	unknown	low molecular weight alcohols	68
1956	MacLean, Heinz	lead oxide, nitrate acetate, basic acetate; magnesium, calcium, barium hydroxides; tin formate	carbohydrate mixture can be hydrogenated to ethylene glycol, glycerol, erythritol. Continuous process	87
1957	Langenbeck, Schwarzer	ion exchange resins	formose	89
1959	Binko, Kolar	MgO	glyceraldehyde, 1,3-dihydroxyacetone	92
1962	Imyanitov	Et_3N + hydrogenation	polyalcohols	100
1965	Skanska Attik- fabriken Aktie- bolag	Ca^{++}, Na^{+}	purification of formose with ion-exchange resins	112
1965	Runge, Mayer	organic bases (e.g. pyridine, collidines)	C_3, C_4, C_5 sugars	114
1970	Berlin	rare earth hydroxide	carbohydrates	134

APPENDIX V

PATENTS COVERING FORMOSE PRODUCTION

The formose reaction can be used as a cheap and efficient means of forming mixtures of polyhydroxyaldehydes and ketones containing up to six carbon atoms. Patents have been taken out in many countries in order to cover various aspects of the processes. They are listed in the Table opposite.

The objectives of the industrial processes are to minimise the extent of the Cannizzaro reaction and caramelisation, to have a high formaldehyde concentration, to have a measure of control over the molecular weight range of the product - and to do this with a small amount of inexpensive, long-life, recoverable catalyst.

As the rate of the Cannizzaro reaction is proportional to the square of the formaldehyde concentration, ways were sought to minimise it while still having a high formaldehyde content. Two methods were found to circumvent this problem - the use of methanol or another alcohol as the solvent instead of water [61, 66, 68] was one, the use of organic bases, like pyridine, in place of the inorganic catalysts was the other.[100, 114] In this way formaldehyde concentrations of up to 20% have been attained. Ion exchange resins have been used as reaction catalysts,[89] but more often the normal inorganic catalysts are employed.

Caramelisation, the other undesirable side reaction, may be reduced by lowering the pH at which the reaction is carried out. In some cases the condensation occurs at pHs below 7,[61, 62, 64, 66] and pH regulation during the reaction proves advantageous.[61, 66]

Lead hydroxide gives a smooth and controllable reaction.[61]

Accelerators are almost always added, as induction periods are expensive. Glucose and fructose, reductone and formose itself are suitable.[62, 64, 82, 114]

The average molecular weight of the products may be selected with some reliability by stopping the reaction at an appropriate time in batch processes, or by changing the reactor residence time in continuous processes.[62,64,66,87,114] Purification of the products by ion-exchange chromatography has been patented.[112,114]

The products of formaldehyde condensation when hydrogenated can give mixtures of polyalcohols.[65,68,87,100] Formose has not been used as a potential source of aldonic acids, perhaps because of the ketose content.

Outwith the formose patents, investigations have been made into continuous processes for formose production.[110,124,127,129,130,133] Some of these are directed towards the use of such systems in closed-circuit respiratory cycles (Appendix VI).

APPENDIX VI

FORMOSE AS A FOOD

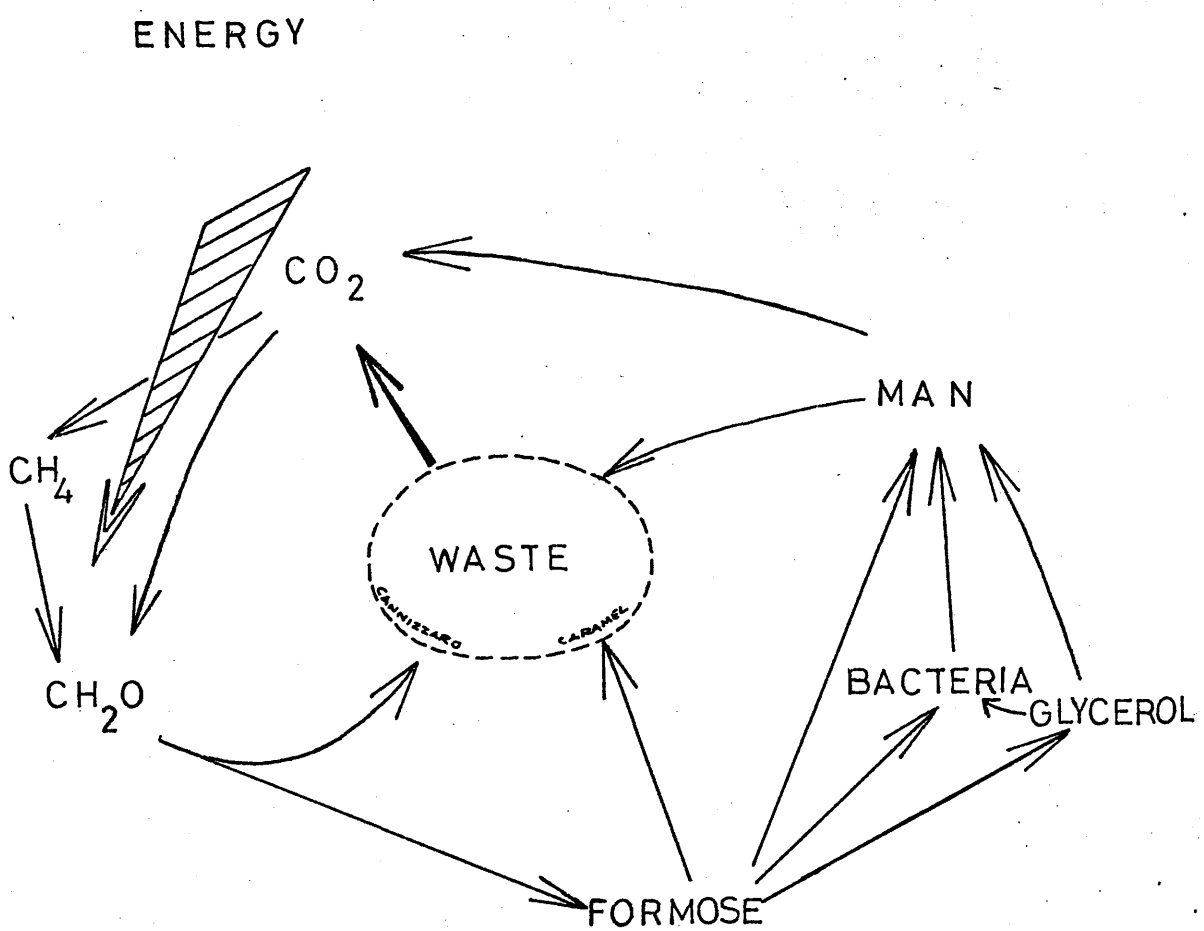
One application of the formose reaction that is gaining in importance is its use as a potential food source, particularly in a closed-circuit respiratory system. Such a system is required if manned spaceflights of a prolonged nature, perhaps to Mars or Venus, are to occur. Weight in those circumstances, is expensive: it would be much better if the food could be re-cycled, with the energy stored in another, more economical form. The re-cycling requires the conversion of carbon dioxide and other waste products to an edible food by the input of energy. The processes involved should be simple, rapid, and not need constant maintenance, complicated apparatus, nor much space. Clearly the formose reaction might satisfy part of this need.

The first stage is the conversion of carbon dioxide and waste to formaldehyde. The preferred method involves the complete reduction of the carbon dioxide followed by partial oxidation.[132,138]

The formaldehyde is then converted into sugar with a suitable catalyst. Several possibilities are then open.[128,130,132] The sugar could be ingested by the astronaut, or it could be used as a food for edible micro-organisms. By reduction the formose may be converted, in part, to glycerol which can be used directly by Man or by the bacteria. A scheme of the alternatives is given on the facing page. (Methods for the reduction of formose are given in Appendix V.)

The first gastronomic observations on formose were made by Butlerow.[2] It was bitter. Loew,[11,12] and Kuzin[49] noted that sweet syrups can result. Normally, however, the tasting of formose is given to small animals, usually rats, which have developed diarrhoea,[110] dehydration, spleen and liver

CLOSED-CIRCUIT RESPIRATORY CYCLE FOR CARBON



atrophy and symptoms of starvation.[138]

Such effects have been shown not to be due to the presence of formaldehyde,[138] nor to the non-physiological L-isomers of the sugars.[132] The effect of branched sugars is unknown.

Formose on its own does not seem to be an ideal food for Man, or for that matter small animals! Micro-organisms may however be able to use the fermentable sugars.[80] There are different reports as to the proportion of fermentable sugar in the formoses produced by the same catalyst, but under different conditions. Nakai found little fermentable sugar in his lime formose,[128] but Ito has shown that only fructose remains after formaldehyde has been in contact with lime at room temperature for 16 days.[116] The maximisation of the yields of particular sugars would probably be a profitable line of research in this field.

Clearly, much work has still to be done before the closed-circuit respiratory systems based on formose are viable.

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